Macro-evolutionary and Macro-ecological Studies on the Cape Flora, with Focus on the *Pentaschistis* Clade (Poaceae)

Dissertation

zur

Erlangung der naturwissenschaftlichen Doktorwürde (Dr. sc. nat.)

vorgelegt der

Mathematisch-naturwissenschaftlichen Fakultät

der

Universität Zürich

von

Chloé A. Galley

aus

Frankreich und Grossbritannien

Promotionskomitee

Prof. Dr. H. Peter Linder (Vorsitz)

Prof. Dr. Elena Conti

Dr. Reto Nyffeler

Zürich, 2007

Acknowledgements

My first and very important thanks go to my supervisor, Peter Linder. I have been inspired by his manner of picking apart problems and data, testing ideas and carrying out novel research. These things helped make the research and this thesis exciting to carry out, and I hope and I think that I have taken some of these skills on board. Additionally, he has a very positive attitude to problem solving which is always welcomed in project work. Through him, I was introduced to the stunning mountains and fascinating vegetation of the Cape and Afromontane Regions, and especially to a curious group of grasses.

Timo van der Niet also played an important role in this project. This stretches from companionship in the mountains of both Africa and Europe, to many discussions, ideas, inspiration and especially support. Chris Hardy likewise was always eager to discuss problems, and helped during field work and also tremendously with analytical issues. Many thanks to: Dirk Bellsteadt and Benny Bytebier especially, for being great co-authors; Philip Moline for help with the GIS; Lukas Taxböck for help with a translation; Frank Rutschmann for help with analytical problems; Urs Landergott for help with statistics; the Zürich Valkyries for keeping me sane, my family for many years of continual support and Merran Mathews and Sam Wüst for much appreciated support and clarifying the texts.

The project was given a head-start by collections of silica plant material from Tony Verboom and Nigel Barker, for which I am very grateful. Fieldwork requires a lot of help from people in the host country. In the Cape, Terry Trinder-Smith and staff at the Bolus herbarium were generous with their time and making me feel welcome there. Also thanks to: Tony Verboom, and Nicola Berge especially, for being excellent ‘field-buddies’ during our adventures in 2004 and for their hospitality; Julia Born and Berit Gehrke for great companionship in the field; Lynn Fish, Clare Archer and Robert Archer from the National Botanic Institute in Pretoria for support and organisational help; Augustin Chikuni and staff at the Botanic Gardens of Malawi, Zomba; Halima and the staff at the herbarium at the National Museums of Kenya, Nairobi; Jacqueline Razanatsoa, Lalao Andriamahefarivo and the staff at the Parc de Tsimbazaza and Missouri Botanic Gardens office in Antananarivo, Madagascar; Pete Phillipson from the Missouri Botanical Garden; Sylvia Philips for assistance at the herbarium at Kew Botanic Garden just before I embarked on the project; and Mary Namanganda and Marc Lebouvier for silica material from places it would have not been possible for me to reach. Lastly, Reto Nyfeller is thanked for dealing the administration relating to my many piles of specimens back in Zurich.

Importantly, I would like to thank the generous sources of funding for supporting my research and conference attendance (the University of Zurich, the Swiss Academy of Sciences and the Science National Fund, grant number 31-66594-01) and fieldwork (Georges und Antoine Claraz-Schenkung).

Lastly, but certainly not least, I thank the people and my friends at the Institute of Systematic Botany, Zurich. It would not have been the same without you all. The working atmosphere in the institute was always supportive, positive and full of interest, from informal meetings and discussions at tea-time, to more formal journal clubs. There were always opportunities to share ideas, ask for advice or technical support and thereby enjoy research and learning.

Table of contents

Introduction

Since it was first botanically explored, the flora of the region at the southern tip of Africa has been known to be special (Goldblatt 1997; Linder 2006). Not only does this Cape flora have high species endemism (69%, Goldblatt & Manning 2002, a level more characteristic of island floras), but it is also incredibly species rich. 9,030 vascular plant species (Goldblatt & Manning 2002) are packed into only 90,000km^2. Such diversity is typical of tropical rainforests or species rich islands, not continental floras so far away from the equator (Linder 2003). The flora has been fairly well collected and documented (Goldblatt 1997), but this is by no means complete; new species continue to be discovered and there is a lot that we can learn about the taxonomy and systematics of the plants.

An important and interesting research challenge is explaining the high species richness of the Cape flora, and one way to approach this is to study the evolution of the plants within the flora (Pennington *et al.* 2004). Studying evolution requires a phylogenetic hypothesis of the clade of interest (Coddington 1988). This allows us to distinguish between shared similarity due to ancestry and that due to convergence. Fortunately, recent advances in obtaining large number of characters (DNA sequence data), and the phylogenetic analysis of these makes it easier to construct a robust phylogenetic hypothesis. Although not without problems (Graur & Martin 2004), analytical advances of these data additionally make a time estimation of events possible. We can start to answer very precisely questions about the 'where' and 'when' of biogeography (Crisp 2006), morphological evolution and the correlated evolution of plant characteristics (Hernandez 2000; Houde 1994; Pagel 1994), the roles of ecology and morphology in lineage diversification (Barraclough & Nee 2001; Schluter et al. 1997), plus the dynamics (Barraclough & Nee 2001; Paradis 1998; Sanderson & Donoghue 1996) and the timing of diversification (Sanderson 2002; Thorne & Kishino 2002). Many of these ideas and analytical methods are new; pushing the boundaries of what we can discover allows us to get closer to explaining how the patterns we observe, were produced.

Accumulating data or information from many of these lineage-specific studies allows us to build up a picture of the whole flora. An important component of the Cape flora is the species-rich Cape clades, which have undergone most of their evolution in the region (Linder 2003). Together, they make up over 50% of the species there. Fortunately, the last decade has seen an incredible amount of research on these clades (Linder 2006) and collaboration should allow ideas relating to the methods and the results to be shared, as well as data synthesis for meta-analysis. The Cape conference held in 2004 in Zurich further promoted this by bringing together many researchers on the Cape flora, and the research presented here was part of this.

This thesis focuses one of these clades, the *Pentaschistis* clade (Danthonioideae, Poaceae) (Barker et al. 2000), which is the most species-rich group of grasses in the Cape flora. It comprises *Pentaschistis* (Nees) Stapf with 70 species, *Pentameris* Beauv. with nine species and *Prionanthium* Desvaux with three species. The clade was named following phylogenetic analysis of DNA sequence data by Barker et al. (2000), and the grouping is supported by morphological, histological and cytological features that are described in chapter one. *Pentameris* and *Prionanthium* are endemic to the Cape Floristic Region (CFR, Goldblatt 1978), but *Pentaschistis* is also distributed in the Afromontane region (White 1983), an archipelago of upland areas in Africa with temperate vegetation. Within the Cape, most of the species are found in a variety of habitats in fynbos vegetation, across wide altitudinal and rainfall ranges and also a range of soil fertility levels. The occurrence of the group in different habitats and geographical areas, and the differences in species richness between these areas, make the *Pentaschistis* clade an ideal group with which to study biogeographical and ecological patterns.

This thesis deals with the systematics, character evolution and taxonomy of the *Pentaschistis* clade, and also examines the role of ecological heterogeneity in explaining the species richness in the CFR. Lastly, I address questions of historical biogeography and differences in species diversity in Africa using multiple lineages. These meta-analyses allow general patterns to be revealed.

In **chapter one** I deal with the evolution of two very interesting characters: multicellular glands, which are unique in the grasses; and the occurrence of two leaf anatomical types. These leaf anatomical types are otherwise evolutionarily conservative in the Danthonioideae, with only one type associated with any genus (Ellis & Linder 1992). What is further interesting is that the two characters are correlated with each other across the clade. The inter- and infra-generic relationships of the *Pentaschistis* clade (Barker *et al.* 2000) have however not been adequately studied, yet this is vital for any evolutionary study.

A species-level phylogenetic hypothesis of the whole clade is constructed, using DNA sequence data from four regions of the chloroplast genome. The phylogeny plus ancestral character state reconstruction are used to investigate the evolution of the glands and their association with leaf anatomical type. Specifically, I ask: How many times did the glands and leaf anatomical types evolve? Which character combinations (leaf anatomical type and gland presence / absence) are associated with diversification? Which lineages tend to lose glands, and do these lineages diversify?

The CFR and the Drakensberg share many species (Goldblatt 1978). Sixty-five species or sub-species of the *Pentaschistis* clade occur in the Cape, and 11 in the Drakensberg. In **chapter two** I make use of this contrasting species richness to understand the high species richness in the Cape, in comparison with the Drakensberg. Species richness is a function of landscape / local-scale richness, and species turnover within the region. Species turnover along geographical and environmental gradients in the Cape is amongst the highest in the world (Cowling et al. 1992; Kruger & Taylor 1979). The richness of landscapes within the Cape also far exceeds that of sub-areas of the Drakensberg. High species turnover may be due to 1) disequilibrium within the species distribution meaning that the species are not as widespread as their habitats would allow them to be; or 2) a turnover in habitats across the Cape, which is then mirrored by species turnover; or 3) more fine division of habitats in the Cape. Explaining landscape richness involves demonstrating whether or not the habitat diversity of landscapes correlates with species richness.

Geographical information system (GIS) is used to match digital macro-environmental data with plant localities. Five variables are used in combination to create bioclimatic ‘points’, which are used to characterise the records and the landscape. The points from the plant records are combined to create bioclimatic envelopes for the species. These bioclimatic envelopes, and the landscape characteristics of the Cape and the Drakensberg are compared. A coarse categorisation method is used, which should help deal with datasets from mega species rich areas, where typically some species are very poorly sampled.

The geographical origins of a flora are the origins of its component lineages. For each lineage, both the place and the time of its origin may be investigated. The origins of the Cape flora are not known, although three hypotheses exist: 1) a tropical African origin followed by southwards migration; 2) an Australian origin; 3) a vicariance (African) origin of the flora. In **chapter three** a meta-analysis is used to test these hypotheses, using phylogenies of the Cape clades. The clades considered form ~30% of the species richness of the Cape (Linder 2003), therefore this meta-analysis synthesises information of a significant proportion of the Cape flora.

For each Cape clade, the phylogenetic positions of extra-Cape species and the distribution of each clade's sister species are used to infer its origin. Phylogenetic trees based on DNA sequence data allow a time estimate of when the lineages entered the Cape. Dates derived from the literature and secondary calibration points are then used to estimate the time over which lineages were recruited into the Cape.

A species may be present in an area because of immigration and / or *in-situ* diversification. To distinguish and compare the roles of these two factors, we need to know the historical biogeography of the lineage. **Chapter four** investigates a component of the Afrotemperate flora in this context. The Afrotemperate Region (Linder 1990; Weimarck 1936; Wild 1964) is an archipelago of isolated areas combining the Afromontane and Cape phytochoria of White (1983). Many of the taxa that are most species-rich in the Cape but not endemic to it, occur in other parts of the Afrotemperate Region. The species richness of these taxa generally decreases eastwards (towards the Drakensberg), and northwards. The routes of migration around the Afrotemperate region are not known. Secondly, although the amount of lineage diversification in the Cape is well known, the diversification in other areas of the region is less well studied.

The biogeographical history of four lineages is reconstructed: the *Pentaschistis* clade, *Disa* (Orchidaceae), Irideae p.p. (Iridaceae) and the African Restionaceae, using phylogenetic trees and likelihood optimisation. Likelihood optimisation allows relative time to be taken into account (as estimated by rate-corrected branch lengths) and uncertainty in the optimisation to be estimated. Additionally DNA phylogenies allow the timing of events to be estimated after calibration of the rate-corrected branches. I ask: in which direction did migration patterns occur? When did migrations occur? In which areas outside of the Cape has local diversification contributed to the species richness, and in which areas has it not?

Linder and Barker (2005) demonstrated that the rate of species description in the Danthonioideae is still increasing. The *Pentaschistis* clade is no exception to this, nor to the on-going alpha taxonomic work in the Cape. **Chapter five** constains the description of three new species and the resurrection of one. Some species have clear characters which distinguish them from other species, as have two of the species described here. In other cases it is more difficult to distinguish whether or not plants should be considered in the same species. Here, one tries to use the taxa or taxon which best reflect(s) the biology, by considering consistent differences between taxa. Two of the new species are such, and ecological and morphological characteristics are found which distinguish the species.

Barker, N. P., Morton, C. M. & Linder, H. P. 2000 The Danthonieae: generic composition and relationships. In *Grasses: systematics and evolution* (ed. J. Everett), pp. 221-230. Melbourne: CSIRO.

Barraclough, T. G. & Nee, S. 2001 Phylogenetics and speciation. *Trends in Ecology & Evolution* **16**, 391-399.

Coddington, J. A. 1988 Cladistic tests of adaptationaly hypotheses. *Cladistics* **4**, 3-22.

Cowling, R. M., Holmes, P. M. & Rebelo, A. G. 1992 Plant diversity and endemism. In *The ecology of fynbos. Nutrients, fire and biodiversity* (ed. R. M. Cowling), pp. 62-112. Cape Town: Oxford University Press.

Crisp, M. D. 2006 Biome assembly: what we know and what we need to know. *Journal of Biogeography* **33**, 1332-1333.

Ellis, R. P. & Linder, H. P. 1992 Atlas of the leaf anatomy in *Pentaschistis* (Arundineae: Poaceae). *Memoirs of the botanical survey of South Africa* **60**.

Goldblatt, P. 1978 An analysis of the flora of Southern Africa: its characteristics, relationships, and origins. *Annals of the Missouri Botanical Garden* **65**, 369-436.

Goldblatt, P. 1997 Floristic diversity in the Cape Flora of South Africa. *Biodiversity and Conservation* **6**, 359-377.
Goldblatt, P. & Manning, J. C. 2002 Plant diversity of the Cape region of Southern Africa. *Annals of the Missouri Botanical Garden* **89**, 281-302.
Graur, D. & Martin, W. 2004 Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends in Genetics* **20**, 80-86.
Hernandez, L. P. 2000 Functional morphology and developmental biology of zebrafish: reciprocal illumination from an unlikely couple. *American Zoologist* **40**, 1054-1054.
Houde, P. 1994 Evolution of the Heliornithidae - Reciprocal Illumination by Morphology, Biogeography and DNA Hybridization (Aves, Gruiformes). *Cladistics-the International Journal of the Willi Hennig Society* **10**, 1-19.
Kruger, F. J. & Taylor, H. C. 1979 Plant species diversity in Cape fynbos: gamma and delta diversity. *Vegetacio* **41**, 85-93.
Linder, H. P. 1990 On the relationship between the vegetation and floras of the Afromontane and the Cape regions of Africa. *Mitteilungen aus dem Institut für Allgemeine Botanik Hamburg* **23b**, 777-790.
Linder, H. P. 2003 The radiation of the Cape flora, southern Africa. *Biological Reviews* **78**, 597-638.
Linder, H. P. 2006 Investigating the evolution of floras: problems and progress - An introduction. *Diversity and Distributions* **12**, 3-5.
Linder, H. P. & Barker, N. P. 2005 From Nees to now - changing questions in the systematics of the grass subfamily Danthonioideae. *Nova Acta Leopoldina* **342**, 29-44.
Pagel, M. 1994 Detecting correlated evolution of phylogenies: a general method for comparative analysis of discrete characters. *Proceedings of the Royal Society of London Series B Biological Sciences* **225**, 37-45.
Paradis, E. 1998 Detecting shifts in diversification rates without fossils. *The American Naturalist* **152**, 176-187.
Pennington, R. T., Cronk, Q. C. B. & Richardson, J. A. 2004 Introduction and synthesis: plant phylogeny and the origin of major biomes. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**, 1455-1464.
Sanderson, M. J. 2002 Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. *Molecular Biology and Evolution* **19**, 101-109.
Sanderson, M. J. & Donoghue, M. J. 1996 Reconstructing shifts in diversification rates on phylogenetic trees. *Trends in Ecology and Evolution* **11**, 15-20.
Schluter, D., Price, T., Mooers, A. O. & Ludwig, D. 1997 Likelihood of ancestor states in adaptive radiation. *Evolution* **51**, 1699-1711.
Thorne, J. L. & Kishino, H. 2002 Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology* **51**, 689-702.
Weimarck, H. 1936 Die Verbreitung einiger Afrikanisch-montanen Pflanzengruppen, III-IV. *Svensk Botanisk Tidskrift* **30**, 36-56.
White, F. 1983 *The vegetation of Africa*. Paris: Unesco.
Wild, H. 1964 The endemic species of the Chimanimani Mountains and their significance. *Kirkia* **4**, 125-157.

The phylogeny of the *Pentaschistis* clade (Danthonioideae, Poaceae) based on cpDNA, and the evolution and loss of complex characters

CHLOE GALLEY AND H. PETER LINDER

Institute of Systematic Botany, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland

Evolution 2007, **61**: 864-884

ABSTRACT

We construct a species-level phylogeny for the *Pentaschistis* clade based on chloroplast DNA, from the following regions: trnL-F, trnT-L, atpB-rbcL, rpL16 and trnD-psbA. The clade comprises 82 species in three genera, *Pentaschistis*, *Pentameris* and *Prionanthium*. We demonstrate that *Prionanthium* is nested in *Pentaschistis* and that this clade is sister to a clade of *Pentameris* plus *Pentaschistis tysonii*. Forty three of the species in the *Pentaschistis* clade have multicellular glands and we use ancestral character state reconstruction to show that they have been gained twice and lost several times. We suggest that the maintenance, absence, loss and gain of glands are correlated with leaf anatomy type, and additionally that there is a difference in the degree of diversification of lineages that have these different character combinations. We propose that both glands and sclerophyllous leaves act as defence systems against herbivory, and build a cost / benefit model where multicellular glands or sclerophyllous leaves are lost when the alternative defence system evolves. We also investigate the association between leaf anatomy type and soil nutrient type on which species grow. There is little phylogenetic constraint in soil nutrient type on members of the *Pentaschistis* clade, with numerous transitions between oligotrophic and eutrophic soils. However, only orthophyllous leaved species diversify on eutrophic soils. We suggest that the presence of these glands enables the persistence of orthophyllous lineages and therefore diversification of the *Pentaschistis* clade on eutrophic as well as oligotrophic soils.

Keywords: gland evolution, ancestral, phylogeny, correlated evolution, *Pentaschistis*, Danthonioideae, Poaceae.

INTRODUCTION

Interest in the evolution of complex structures and their influence upon the success of organisms has a long history. Classic examples include orchid flowers (Darwin 1862) and the evolution of eyes (Fernald 2004). Part of their fascination lies in the very low probability that such structures should evolve at all (Dawkins 1986). However, their (often) unique evolution makes it difficult to tease apart the adaptive aspects from other factors, including chance. In contrast, the loss of such complex structures presents us with an opportunity to explore their function. Losses tend to be more numerous than gains in many complex structures or traits (e.g. heterostyly (Kohn et al. 1996; Schoen et al. 1997); secondary xylem in aquatic plants (Sculthorpe 1967); wings in insects (Whiting et al. 2003); or the chlorophyll producing function of chloroplasts, in holoparasites (Judd et al. 2002; APG 2003; Bungard 2004; Nickrent et al. 2004)). This makes them more tractable to investigation than gains. For example, if the losses of a particular structure within a clade are non-random with respect to other morphological characters or habitat, these losses can be used to investigate the function of this structure. Here we investigate the evolution of multicellular glands in the *Pentaschistis* clade in this context.

Peculiar multicellular glands (see Fig. 1a, b) occur on the inflorescences and / or leaves in forty-three species of *Pentaschistis* and of *Prionanthium*, and are formed from groups of cells, of which some are secretory. These glands are unique in the grasses, developing from the epidermal cells, rather than from pre-existing bicellular hairs on the epidermis (Davidse 1988; Linder et al. 1990). They range from simple linear glands consisting of enlarged epidermal cells arranged in a series, to more elaborate glands that have a greater size differentiation between glandular and epidermal cells, and further some that have differentiated cell types within the gland. Equating the elaborate type with a derived condition (and ‘simple’ as plesiomorphic), evolutionary progression from simple to complex glands was suggested following morphological-anatomical analysis (Linder et al. 1990). However neither the evolutionary progression nor the number of times that glands were gained or lost has been thoroughly investigated. To date no species-level phylogenetic hypothesis of the clade exists. The current infra-generic classification of *Pentaschistis* was based on morphological data and was constructed to aid communication and identification, not to reflect phylogeny (Linder & Ellis 1990). To understand the evolution of these glands, however a historical, phylogenetic component is vital (Felsenstein 1985b; Pagel & Harvey 1988), preferably one based on

characters other than those being studied (Coddington 1988; Armbruster 1992; Luckow & Bruneau 1997).

The *Pentaschistis* clade (Barker et al. 2000) comprises *Pentaschistis* (Nees) Stapf with ~70 species, *Pentameris* Beauv. with nine species and *Prionanthium* Desvaux with three species, and is the most species-rich group of grasses in the Cape flora of South Africa. Although *Pentameris* and *Prionanthium* are both endemic to the Cape Floristic Region (CFR, Goldblatt 1978), *Pentaschistis* is widespread in temperate habitats in sub-Saharan Africa and is a frequent element in the Afromontane and Afroalpine vegetation (Knapp 1973). Within the CFR, most of the species are found in a variety of habitats in fynbos vegetation across wide altitudinal and rainfall regimes. They occur mostly on soils derived from sandstones of the Cape Supergroup, but also on the limestone hills of Bredasdorp (e.g. *Pentaschistis calcicola*), coastal sands (e.g. *Pentaschistis barbata*), shale in coastal Renosterveld (e.g. *Prionanthium ecklonii*) and silcrete derived soils (e.g. *Pentaschistis juncifolia*).

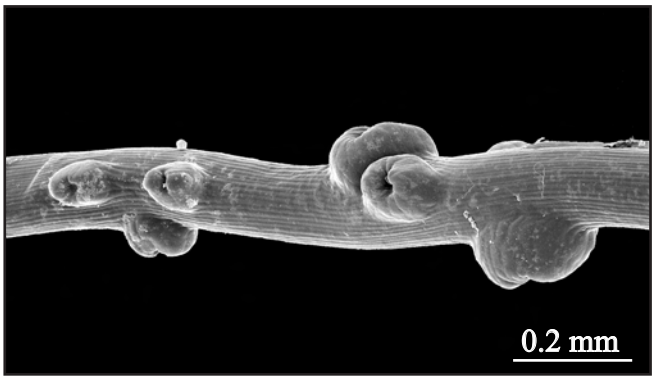
This essentially south-temperate group (Linder 1989) is taxonomically well known (Linder & Ellis 1990; Phillips 1994; Galley & Linder 2006). The cytology of many species has been investigated, all three genera have a basic chromosome number of x = 7 (Davidse 1988; du Plessis & Spies 1992; Spies & Roodt 2001). Comprehensive investigation of the leaf anatomy of almost all species in the clade has been carried out (Ellis 1989; Ellis & Linder 1992; Barker 1993) allowing a broad classification of the species into two types, those with orthophyllous leaves and those with sclerophyllous leaves (Ellis & Linder 1992). Micro-morphological characteristics used to define these leaf types are shown in Table 1 and illustrated in Figures 1b and 1c. Orthophyllous leaves tend to have soft blades and a life-expectancy of at most one growing season, whereas sclerophyllous leaves are thick and tough, generally living for more than one growing season (Ellis & Linder 1992).

The evolution and function of the glands however remains a puzzle, and we seek to account for why some species have these complex glands whereas others do not. We reconstruct a species-level phylogeny of the *Pentaschistis* clade based on plastid DNA sequence data to resolve phylogenetic relationships within the clade, and to determine how many times glands evolved and have been lost. It is evident that there is a relationship between the presence of these glands and leaf anatomy type within the clade: glands are more common in orthophyllous taxa than in sclerophyllous taxa (Ellis & Linder 1992). The evolution of these glands is therefore most productively addressed in relation to leaf anatomy type. We use ancestral character state reconstruction to investigate the frequency with which character combinations evolve and are retained, and also compare lineage diversification

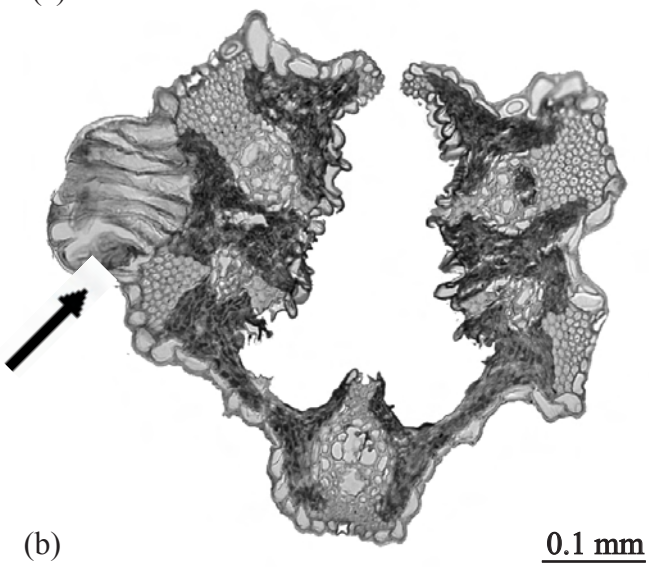
between these character combinations. Finally, we seek to relate leaf anatomy type and soil nutrient type.

Table 1. Characteristics of sclerophylls and orthophylls, adjusted from Ellis and Linder (1992)

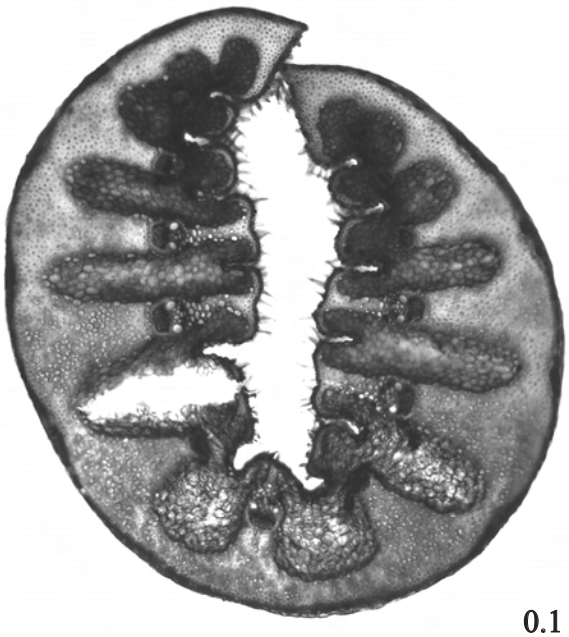
orthophyllous leaves	sclerophyllous leaves
Diffuse mesophyll of large rounded parenchyma cells	Compact mesophyll of small isodiametric cells
Extensive intercellular air space system	Minute intercellular air spaces
Sclerenchyma only associated with the bundles as girders or strands	Sclerenchyma abundant---often continuous between bundles (via the hypodermis)
Cuticle and outer walls of epidermal cells thin	Cuticle plus outer epidermal cell wall thickened
Abaxial stomata present	Abaxial stomata absent
Epidermal zonation evident (abaxial)	No distinction between costal and intercostal zones in surface view



(a)



(b) 0.1 mm



(c) 0.1 mm

Figure 1. a. Stalked multicellular gland from *Pentaschistis airoides* subsp. *jugorum*; b. Transverse section (TS) of a leaf of *Pentaschistis clavata*, illustrating the orthophyllous anatomy type with non-continuous girders (pale grey) and mesophyll tissue (dark grey), also a multicellular gland (arrow); c. TS of a leaf of *Pentashistis horrida*, illustrating the sclerophyllous anatomy type with extensive, continuous girders (pale grey) and restricted mesophyll tissue (dark grey).

MATERIALS AND METHODS

Sampling

We attempted to obtain complete species sampling for the *Pentaschistis* clade, based on the taxonomy of Davidse (1988), Linder and Ellis (1990) and Barker (1993), as well as recently described species and taxonomic changes (Phillips 1994;1995; Galley & Linder 2006). Seventy-three out of 82 species were obtained, representing 80 out of 90 taxa, including the four varieties of *Pentaschistis pictigluma* and both sub-species of *P. airoides* and *P. aurea*. Based on the sub-family phylogeny of Barker et al. (2000), two *Merxmuellera* species were selected as out-groups. Material was collected into silica gel in the field, except for material from three species which was obtained from herbarium specimens (see Appendix 1 for details).

Molecular Techniques

Tissue collection and DNA isolation

DNA was extracted using DNeasy Plant Mini Kits (Qiagen, Switzerland) following the manufacturer's protocol or the CTAB method (Doyle & Doyle 1987). Where DNA was extracted from herbarium material a modification of the SDS method (Eichenberger and Schneller, 2000) of DNA extraction was used, repeating the chloroform cleaning step. Polymerase chain reactions (PCRs) were performed in a Biometra Thermocycler TGradient (Biometra, Göttingen, Germany) using a total reaction volume of 25 µL with 2.5 mM $MgCl_2$, 1xPCR buffer (Amersham Biosciences, Otelfingen, Switzerland), 0.25 mM dNTPs, 1.6 µM primer and 1 unit of Taq polymerase (Amersham Biosciences, Otelfingen, Switzerland, and Sigma-Aldrich, USA). Additives were used as described. The intergenic spacers *trnT-L, L-F, atpB-rbcL*, *trnD-psbA* and introns of *trnL* and *rpL16* were amplified by the PCR method and sequenced using the primers as shown in Table 2.

Table 2. Primers used for PCR and sequencing

gene region	primers (PCR)	primers (sequencing)	source	sequence (if from this study)
trnL-F	c,f	c,d,e,f, c2 d3	(Taberlet et al. 1991) this study this study	 5'-GGT CCT YAA ACT ARA ACC C-3' 5'-GKG KMT RGT ATT ATA TCC-3'
trnT-L	a,b	a,b	(Taberlet et al. 1991)	
atpB-rbcL	f1c, r1a2	f1c, r1a2 atpBrbcL_Fint atpBrbcL_Rint	Hardy and Linder 2005 this study this study	 5'-GTG TAY TGK ACR TTC TA-3' 5'-CCR AAA WYC CAA ARG CCA-3'
rpL16	F71 R1000	F71 R1000	(Baum et al. 1998) this study	 5'-CTG TTC TTT TRG GTT ATA GTC-3'
trnD-psbA	trnD psbM	trnD psbM trnCD3 trnCDF2	(Shaw et al. 2005) (Shaw et al. 2005) this study this study	 5'-TAG AGT WCC MRT ATT TTA CCG-3' 5'-CTA GGG TTC AAT GAA TGG-3'

PCR amplification

The *trnL-F* intergenic spacer and *trnL* intron (trnLF) were amplified using a PCR protocol with an initial denaturation step of 94°C for 3 mins followed by a step-down program with 4 cycles of 1 min denaturation at 94°C, 1 min annealing at 56°C, 1 min extension at 72°C, then 28 cycles of 1 min at 94°C, 1 min at 52°C and 1 min at 72°C, terminated by a final extension of 7 mins at 72°C. The *trnT-L* intergenic spacer (trnTL) was amplified using an initial denaturation step of 94°C for 3 mins followed by a step-down program with 4 cycles of 1 min at 94°C, 1 min at 54°C, 1 min at 72°C, then 28 cycles of 1 min at 94°C, 1 min at 52°C and 1 min at 72°C, terminated by a final extension of 7 mins at 72°C. 1 µL of BSA was used in all reactions. The *atpB-rbcL* intergenic spacer (atpB-rbcL) was amplified using an initial denaturation step of 95°C for 3 mins followed by a step-down program with 3 cycles of 1 min at 95°C, 1 min at 55°C, 1 min at 72°C, then 6 cycles of 1 min at 95°C, 1 min at 52°C, 1 min at 72°C, then 26 cycles of 1 min at 94°C, 1 min at 48°C and 1 min at 72°C, terminated by a final extension of 7 mins at 72°C. 1.2 µL DMSO or 4 µL of BSA were used for several samples that would otherwise not amplify. The *rpL*16 intron (rpL16) was amplified using an initial denaturation step of 94°C for 4 mins followed by 34 cycles of 1 min at 94°C, 1 min at 55°C and 1 min 20 secs at 72°C, terminated by a final extension of 7 mins at 72°C. 1 µL BSA was used in each sample. The *trnD-psbA* intergenic spacer (trnD) was amplified using an initial denaturation step of 94°C for 3 mins followed by 30 cycles of 1 min at 94°C, 1 min at 52°C and 1 min 40 secs extension at 72°C, terminated by a final extension of 7 mins at 72°C. 1 µL of BSA was used for several samples that would otherwise not amplify. All PCR products were visualised on a 1.5% agarose gel. Double

bands were found for some accessions for trnD. In these cases the band of appropriate size (between 1,100 and 1,200 base pairs) was excised and purified with a DNA band purification kit (Amersham Biosciences, USA) before sequencing.

Sequencing

PCR products were cleaned using the QIAquick PCR purification kit (Qiagen, Basel, Switzerland) according to the manufacturer's protocol and cycle sequencing was carried out on a ABI Prism 3100 Genetic Analyzer (Applied Biosciences, Foster City, USA) using BigDye terminator versions 2.0 and 3.1 without and with 5x buffer (Applied Biosciences, Foster City, USA), respectively.

Phylogenetic Analysis

Data matrices were aligned by eye and gaps were coded by hand using the nested gap principle of Simmons and Ochoterena (2000), except for gaps created by poly A or poly A/T regions and gaps that represented single base pairs, which were not coded. Datasets were analyzed separately and in combination using the parsimony criterion, by means of the Ratchet implemented in Winclada (Nixon 1999-2002). Default settings were used, calculating 12 consecutive runs. Bootstrap support (BS) (Felsenstein 1985a) was also calculated in Winclada using 500 bootstrap replicates with 50 replicates per bootstrap replicate, holding three trees per replicate. Potential incongruence between datasets was assessed visually by comparing 1. phylogenies from individual datasets and 2. the support and resolution of phylogenies from the combined data versus individual gene regions. The only incongruence with BS above 65% is *Pentameris macrocalycina* which groups with three different taxa in different gene regions, and is supported in each of these places with 82%, 91% and 97% BS. The combined 'chloroplast' dataset resulted in a more highly resolved phylogenetic tree with greater support for the groupings than did the individual datasets, and was used for final phylogenetic analysis.

The chloroplast dataset was additionally analyzed with a likelihood criterion using Bayesian analysis implemented in MrBayes 3.0 beta 4 (Huelsenbeck & Ronquist 2001). The dataset was separated into seven partitions (rpL16, trnTL, trnD, trnLF, atpB-rbcL, the poly-AT regions, gapcoding). Poly-AT regions and gap coding from all gene regions were pooled to form the 'poly AT' and 'gaps' partitions, respectively. The seven partitions were analyzed

individually or combined, to test whether pooled partition 'schemes' might yield a better fitting model. All partitions were analyzed separately in the seven-partition scheme, gene regions (excluding poly AT data) were merged in the three-partition scheme, and all DNA characters were merged in the two-partition scheme (see Table 3). Each partition scheme (except for 'gapcoding', which was coded as 'standard' data for the phylogenetic analysis) was assigned a model using Modeltest (Posada & Crandall 1998) implemented in PAUP* (Swofford 2002), using the Akaike Information Criterion (AIC, Akaike 1973) to choose between models. Information from the model (Nst, gamma and presence of invariant sites) was then used in the Bayesian analysis, but the parameter values were free to vary. Each of the three partition schemes was analyzed using Bayesian phylogenetic analysis with four chains (three hot, one cold) run for 4,000,000 generations sampling every 1,000 generations. The likelihood values of the sampled models were checked in previous shorter analyses (500,000 generations) to obtain burnins of 170,000 (two and three partition analysis) and 250,000 (seven partition analysis) generations. The likelihood of each parameter was checked for stability, indicating convergence.

The harmonic mean of log likelihoods (lnL) of all generations (excluding burn-in) was used to compare the three partition schemes, as this is less sensitive to outliers than the mean lnL (Nylander et al. 2004). The AIC was used to compare these harmonic means as it penalizes high-parameter models (Burnham & Anderson 2004). The 3-partition model had a AIC score that was 243 units higher than the next competing model set (see Table 4), a score considered to provide strong evidence (Burnham & Anderson 2004). Consequently the 3-partition model was used for further analyses.

Table 3. Partition schemes tested in Bayesian analysis and results from the AIC test.

	Partitions	Parameters	lnL of the harmonic mean	AIC	Δ AIC
All DNA (1) gaps (1)	2	14	-21978.24	19483.68	338
gene regions (1) poly AT (1) gaps (1)	3	26	-21957.59	19145.96	0
gene regions (5) poly AT (1) gaps (1)	7	62	-2283.96	19389.30	243

We checked three subsequent runs for the convergence of topology and likelihood scores. From all the runs, the generation that received the overall highest likelihood score was used as a 'final topology'. This overall likelihood score is a combination of all parameters of

which the topology is just one. However, a fully resolved topology was preferred for ancestral state reconstruction and we find this method of choosing a topology superior to randomly picking one. A 50% majority rule of the set of sampled trees (excluding burn-in trees) was constructed to obtain posterior probabilities (p.p.) as a measure of node support. Nodes which were not in this 50% set were treated as described below.

Following rejection of a molecular clock (df = 86, P = 0.05) branch lengths were made ultrametric using Penalized Likelihood in r8s (Sanderson 2002;2003) using the smoothing parameter 0.00079, yielded from cross validation.

Description and Scoring of Glands and Leaf Anatomy Type

Glands were scored as either 1. absent 2. linear or 3. rounded, based on personal observation and literature (Davidse 1988; Linder & Ellis 1990; Linder *et al.* 1990; Barker 1993). Linear type glands were scored as such, and the club shaped, sessile elongated and sunken crateriform glands (see Linder et al. 1990) were scored as rounded. We did not consider the position of the glands on the plant, assuming that their function is independent of their position.

Data on leaf anatomy type were mostly derived from the literature (Ellis 1989; Ellis & Linder 1992; Barker 1993) and species were classified as 1. sclerophyllous or 2. orthophyllous, in accordance with Ellis and Linder (1992). The leaf anatomy type is inadequately known for *Pentaschistis andringitrensis* A.Camus, *P. insularis* (Hemsl.) Linder, *P. clavata* Galley, *P. horrida* Galley and *P. trifida* Galley. Hand- and microtome- sections were prepared from herbarium specimens for these species. For the hand-sections, midsections of dried leaves were softened in boiling soapy water for 10-15 minutes, hand-sectioned, and stained in a 1:1 mix of safranin red / alcian blue (Tolivia & Tolivia 1987) for 5-10 minutes. After rinsing in water the sections were dehydrated in ethanol and mounted with histoclear and histomount. For the microtome-sections, midsections of dried leaves were softened in sulfosuccinate sodium salt and acetone for 2-5 hours, plastic embedded in GMA (Igersheim & Cichock 1996) and sectioned to 6-8µm thick. Sections were stained in toluidine blue (3-4 mins) and ruthenium red (1-2 mins) and mounted with histoclear and histomount. Leaf anatomy type was assigned according to Ellis and Linder (1992) using the characters shown in Table 1.

Scoring of Soil Nutrient Type

Each species was assigned to either i. eutrophic or ii. oligotrophic soil type based on the bedrock or substrate type on which the species grows. This information was obtained from herbarium specimen label data (BOL and Z collections) and from field observations. Eutrophic soil type was assigned to soils derived from shale, coastal sands, granite, silcretes and basalt, and oligotrophic soil type was assigned to soils derived from sandstones of the Cape Supergroup (CS), limestone (pavements), cave sandstone and well-leached (acid) sands. Soil fertility is difficult to quantify, dependant not only on the nitrogen (N) and phosphorus (P) status of the soils, but also their availability. We base our assignments on literature (Killick 1979; Kruger 1979; Lambrechts 1979; Witkowski & Mitchell 1987) using P and where possible, N levels as guides. Likelihood optimization does not allow polymorphic taxa. In the few cases that a species is recorded from both eutrophic and oligotrophic soils, it was scored only as oligotrophic as such nutrient constraints would be expected to be a limiting factor for the plants.

Character Optimization

A likelihood approach was used for ancestral character state reconstruction, which enables the rate corrected branch lengths to be taken into account. More importantly, it provides an estimate of the uncertainty in the reconstruction (Schluter et al. 1997), which is especially relevant for nodes deep in the phylogeny. Branches receiving less than 0.50 p.p. were shortened to a length of 0.000001. All nodes were optimized in Mesquite ver. 1.11 (Maddison & Maddison 2006). A two-rate model offered no significant improvement in likelihood for leaf anatomical type, so following Mooers and Schluter (1999), a one-rate model was used. A two-rate model did offer significant improvement in likelihood for soil fertility and gland presence and was used for these characters. A difference of two log likelihood units (lnL) between character states was considered as statistically significant (Pagel 1994;1999) and these nodes were optimized as such. Nodes that could not be optimized with statistical significance were omitted from further calculations, or in the case of soil fertility were optimized with the state receiving the highest likelihood (if support was above proportional likelihood of 0.80; see Results). In all cases out-groups were removed from the phylogenetic tree and the tree was rooted at the node between the *Pentameris s.l.* clade and *Pentaschistis* / *Prionanthium* clade.

Taxa were scored with binary coding and all internal nodes were optimized for glands as a) 1. present 2. absent, b) 1. linear 2. not linear (i.e. no glands, or rounded glands) and c) 1. rounded 2. not rounded (i.e. no glands or linear glands); for leaf anatomy type 1. orthophyllous 2. sclerophyllous; and for soil type 1. eutrophic 2. oligotrophic.

Associations between characters

A modification of the Contingent States Test (Sillén-Tullberg 1993; Werdelin & Tullberg 1995) was used to test for the association between character states, as this test allows both the maintenance and the change in character states to be considered separately. For each node two 'events' are counted, i.e., from the node to each of the daughter lineages (Sillén-Tullberg 1993). For each event the maintenance or change in character state for both characters is scored as illustrated in Figure 2.

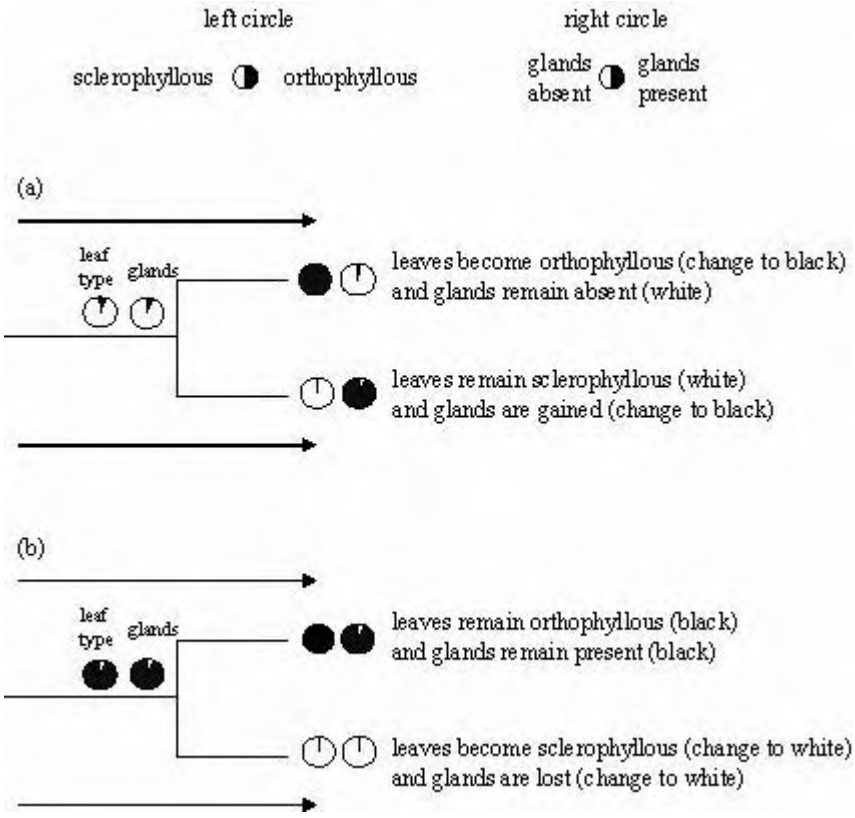


Figure 2. Illustration of how 'events' are counted at each node according to the contingent states test (Sillén-Tullberg 1993). Glands may remain absent or evolve (a) or they may persist or be lost (b). Likewise leaves may remain orthophyllous or sclerophyllous or transform between types. 16 types of events are possible.

There are two cases of topological uncertainty which might alter the changes inferred, depending on which way they are resolved. Although these do not alter the overall conclusions we reach, these nodes were omitted from the count in changes (indicated by

arrows in Fig 5). To examine the association between gland evolution and leaf anatomy type we scored the number of cases of 1. maintenance of glands, 2. maintenance of no glands, 3. evolution of glands and 4. loss of glands, against two leaf types 1. orthophyllous, including transformation from sclerophyllous to orthophyllous, and 2. sclerophyllous, including transformation from orthophyllous to sclerophyllous. To examine the association between leaf anatomy type and soil nutrient type we tested the different leaf states (as above) on 1. oligotrophic, including change from eutrophic to oligotrophic, and 2. eutrophic, including change from oligotrophic to eutrophic soil types. The soil types were then decomposed to investigate the diversification associated with a transition between soil types and diversification within soil each type. A G-test using Yates's correction for small values was used to test the significance of these associations (Sokal & Rohlf 1995). In several cases multiple accessions were left in the phylogeny to represent the different lineages but for the Contingent States Tests one accession of *P. natalensis* and *P. colorata* each was omitted.

RESULTS

Phylogeny Reconstruction

A matrix with a total of 5500 aligned characters, combining DNA sequence data and 56 gap characters from five data partitions representing four chloroplast regions, was analyzed. The number of parsimony informative characters for atpB-rbcL was 109, for rpL16, 104, for trnLF, 130, for trnTL, 179 and for trnCD, 135. There are no nodes in the strict consensus of the most parsimonious trees that conflict with the 50% majority rule set of Bayesian trees. There is a general positive relationship between the BS and Bayesian p.p. values but some nodes that have 1.00 Bayesian p.p. values have a wide range of BS.

Clade names have been allocated to groups in the phylogeny for convenience, and are named as the first species to branch off within the group. *Pentameris* plus *Pentaschistis tysonii* form a clade (*Pentameris s.l.* clade, I) that is sister to the remainder of the *Pentaschistis* clade including *Prionanthium* (see Fig. 3), the '*Pentaschistis* / *Prionanthium* clade'. The monophyly of each of these clades is strongly supported. Within the large *Pentaschistis* / *Prionanthium* clade the *P. elegans* clade (II) is sister to the rest of the group. The next species to branch are *Pentaschistis basutorum*, *P. juncifolia* and *P. andringitrensis*, forming a grade. The remaining species form a well supported clade containing almost all of the glanded taxa. The first clade to branch within this is the *P. argentea* clade (III), then the *P.*

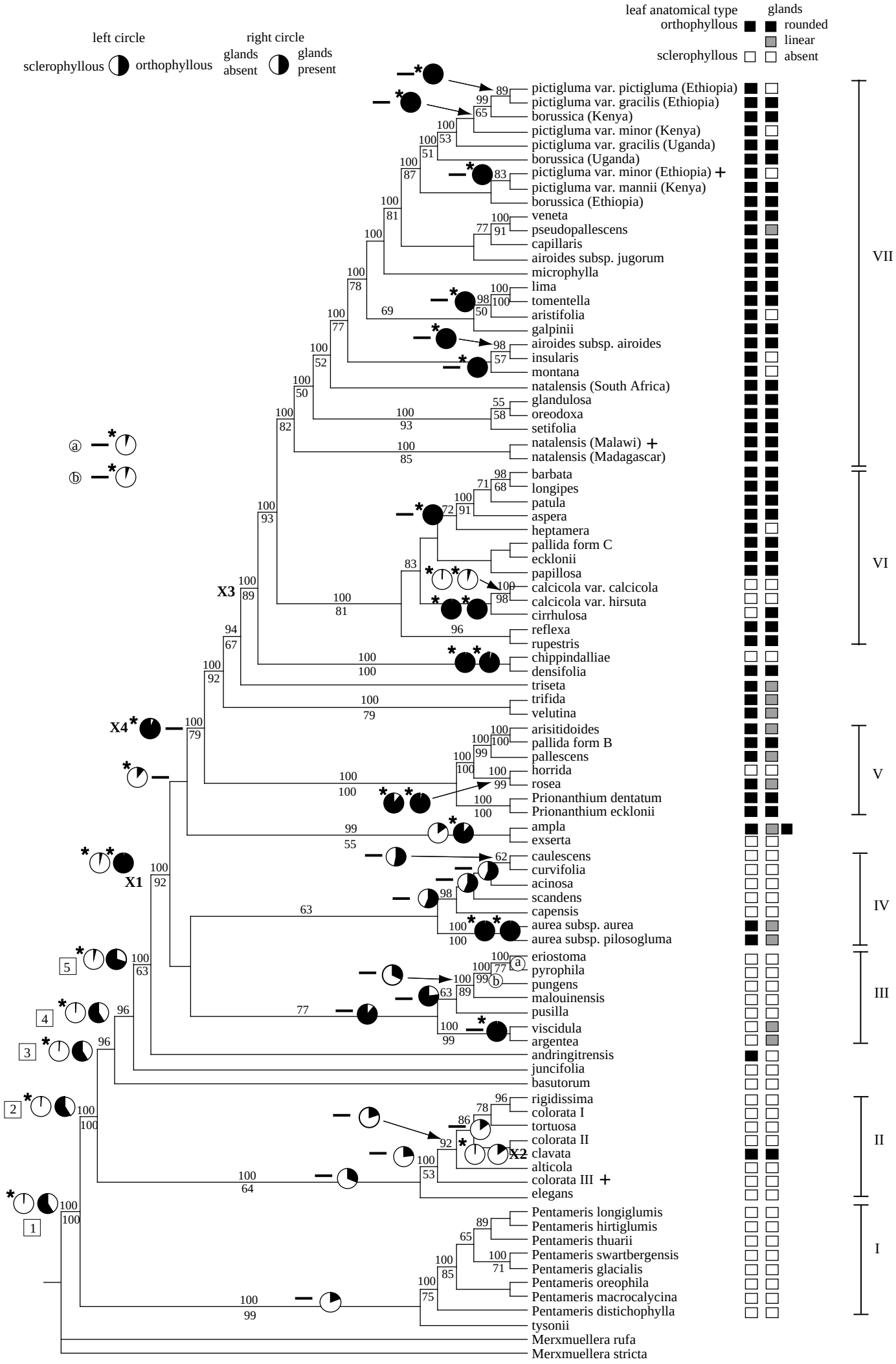
aurea clade (IV). The *P. ampla* and *P. exserta* species pair are next to branch. The two *Prionanthium* species sampled are sister to the *P. rosea* group and this strongly supported clade (V) is the next clade to diverge. Two species pairs and *P. triseta* form a grade that diverges basally to a well supported clade that comprises a 'coastal clade' (VII) and a large 'summer rainfall clade' (VI). The 'coastal clade' comprises two non-coastal species (*P. reflexa* and *P. rupestris*) but the core group is distributed near the coast, albeit growing on soils derived from a variety of rock types. The 'summer rainfall clade' contains species occurring in the drier areas (*P. lima*, *P. aristifolia*, *P. tomentella*, *P. airoides* ssp. *jugorum*, *P. pseudopallescens*, *P. veneta* and *P. montana*) but also most of the summer rainfall species. These include several species distributed in the Drakensberg (South Africa), a non-monophyletic *P. natalensis* ranging from the Drakensberg north towards southern Tanzania and east to Madagascar, and the taxa that occur exclusively in the mountains of tropical Africa.

Evolution of Glands and Leaf Anatomy Type

We classify the leaf anatomy type of *Pentaschistis clavata*, *P. andringitrensis*, *P. insularis* and *P. trifida* as orthophyllous, and *P. horrida* as sclerophyllous.

In the ancestral character state reconstruction of gland presence / absence, almost all nodes were unambiguously assigned a character state, except some nodes along the spine of the phylogeny (see Fig. 3, nodes 1-5) and other nodes as shown (see Fig. 3). These nodes were omitted from further calculations because the likelihood values between character states for the glands and / or leaf anatomy type were almost equivocal.

Figure 3. (following page) = Phylogenetic hypothesis from Bayesian analysis showing the topology from the most likely model, with posterior probabilities from the Bayesian analysis (> 50 support) shown above branches (probabilities 0 to 1 converted to scores 0 to 100) and bootstrap support (BS) support below branches (> 50% support). The left column of squares shows taxa as orthophyllous or sclerophyllous and the right column of squares shows taxa as having no glands, linear glands or rounded glands. Pie charts show optimization (as proportional likelihoods) at nodes that subtend gains / losses in glands, also nodes that are unambiguously optimized. Pie charts are marked with an asterisk when optimization is statistically significant. Dashes are placeholders for pie charts that are not shown. Node X1 and tip X2 indicate points by which glands have evolved. Node X3 is where the rounded glands are reconstructed as having evolved from linear glands. Unless otherwise illustrated all nodes within this glanded clade (X1) were optimized with statistical significance as glands 'present', and all nodes outside it as glands 'absent'. Unless otherwise illustrated all nodes in clade X4 were optimized with statistical support as orthophyllous leaved and nodes outside this clade as sclerophyllous. Accessions marked with a +, and nodes numbered in square boxes were omitted from the Contingent States Test (see text). Clade names (I to VII) are informal names referred to in the text. Unless otherwise indicated all species are *Pentaschistis*.


left circle
sclerophyllous
orthophyllous
right circle
glands absent
glands present
leaf anatomical type
orthophyllous
sclerophyllous
glands
rounded
linear
absent
pictigluma var. pictigluma (Ethiopia)
pictigluma var. gracilis (Ethiopia)
borussica (Kenya)
pictigluma var. minor (Kenya)
pictigluma var. gracilis (Uganda)
borussica (Uganda)
pictigluma var. minor (Ethiopia) +
pictigluma var. mannii (Kenya)
borussica (Ethiopia)
veneta
pseudopallescens
capillaris
airoides subsp. jugorum
microphylla
lima
tomentella
aristifolia
galpinii
airoides subsp. airoides
insularis
montana
natalensis (South Africa)
glandulosa
oreodoxa
setifolia
natalensis (Malawi) +
natalensis (Madagascar)
barbata
longipes
patula
aspera
heptamera
pallida form C
ecklonii
papillosa
calcicola var. calcicola
calcicola var. hirsuta
cirrhulosa
reflexa
rupestris
chippindalliae
densifolia
triseta
trifida
velutina
arisitidoides
pallida form B
pallescens
horrida
rosea
Prionanthium dentatum
Prionanthium ecklonii
ampla
exserta
caulescens
curvifolia
acinosa
scandens
capensis
aurea subsp. aurea
aurea subsp. pilosogluma
eriostoma
pyrophila
pungens
malouinensis
pusilla
viscidula
argentea
andringitrensis
juncifolia
basutorum
rigidissima
colorata I
tortuosa
colorata II
clavata
alticola
colorata III +
elegans
Pentameris longiglumis
Pentameris hirtiglumis
Pentameris thuarii
Pentameris swartbergensis
Pentameris glacialis
Pentameris oreophila
Pentameris macrocalycina
Pentameris distichophylla
tysonii
Merxmuellera rufa
Merxmuellera stricta
VII
VI
V
IV
III
II
I
X1
X2
X3
X4
a
b
1
2
3
4
5

Glands evolved twice in the *Pentaschistis* clade: once in *Pentaschistis clavata* and once at the base of a larger clade comprising at least 58 species (see Fig. 3, X2 and X1, respectively). At node X1 there is strong evidence of glands being present (difference in lnL of 3.34), whereas the node preceding this is much less decisive (difference in LnL of 0.23). There are nine nodes between X1 and *P. clavata*. Although only three of these optimize significantly as 'absent' for glands, forcing the optimization to a single gain of glands, would require eight losses of glands in the eight diversification events between them. Based on our sampling, the most likely hypothesis is therefore that glands evolved twice: once as rounded glands (*P. clavata*) and once as linear glands (Fig. 3, X1). Rounded glands evolved from linear glands four times, including *P. ampla*, *Prionanthium*, *P. pallida*, and by node X3 (see Fig. 3), but there is only a single transition in the reverse direction (*P. pseudopallescens*). There are several losses of glands (see Fig. 4), from both linear and rounded glands. Unequal rates of gland gain and loss are also demonstrated by likelihood analysis. The significant difference between the single-rate and the two-rate models for glands (difference in lnL = 4.3) shows that the difference between the rates of gland gain (0.517) and loss (2.852) is significant.

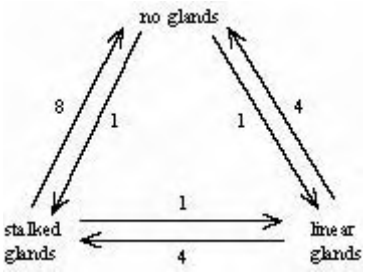


Figure 4. Number of changes between the gland states.

We found a significant bias in the distribution of glands relative to leaf type (G-test, df = 1, P < 0.000). Glands occur more frequently in species that have orthophyllous leaves, whereas eglandular species tend to have sclerophyllous leaves.

In both cases glands evolved near or at a change from sclerophyllous to orthophyllous leaf type and a total of 12 gland losses occur in the *Pentaschistis* clade (the losses in the two geographic lineages of *Pentaschistis pictigluma* var. *minor* are counted as one event). The maintenance of a glandless state is higher in a background of sclerophyllous leaves than orthophyllous leaves (Table 4). Some of this bias derives from the evolution of glands in a part of the lineage where few of the sclerophyllous species occur. However, this bias remains if we consider species within the glanded clade only; three of the sclerophyllous lineages that have lost glands diversify and these lineages all remain glandless, representing 16

diversification events. In contrast to this none of the six orthophyllous lineages that lose glands diversify. There is a strong bias in gland maintenance and gland loss between the different leaf types (see Table 4; G-test, df = 1, P = 0.005) and the ratios of gland maintenance to gland loss are ~ 12 : 1 for orthophyllous lineages and ~1: 1 for sclerophyllous lineages. This demonstrates that glands tend to be kept rather than lost in orthophyllous lineages. There are only three taxa that have both glands and sclerophyllous leaves, including one diversification event.

Table 4. Number of 'events' counted as for the Contingent States Test. Glands can either remain absent (0 to 0) or they can evolve (0 to 1) against a background of orthophyllous (0) or sclerophyllous (1) leaf type. Glands can remain present (1 to 1) or they can be lost (1 to 0) as shown, against a background of orthophyllous (0) or sclerophyllous (1) leaf type.

		glands			
		0 to 0	0 to 1	1 to 1	1 to 0
leaf anatomy type	orthophyllous (0 to 0 and 1 to 0)	0	1	88	7
	sclerophyllous (1 to 1 and 0 to 1)	29	1	5	4

In sum, the two character combinations sclerophyllous leaves and no glands, and orthophyllous leaves and glands are associated with a high number of diversification events. In contrast the two character combinations sclerophyllous leaves with glands, and orthophyllous leaves without glands are associated with few or no diversification events, respectively.

There are nine transitions between orthophyllous and sclerophyllous leaf type in the *Pentaschistis* clade (see Fig. 3). There are four transitions from orthophyllous to sclerophyllous leaf type, three of which are accompanied by the loss of glands.

Soil Nutrient Status and Leaf Anatomy

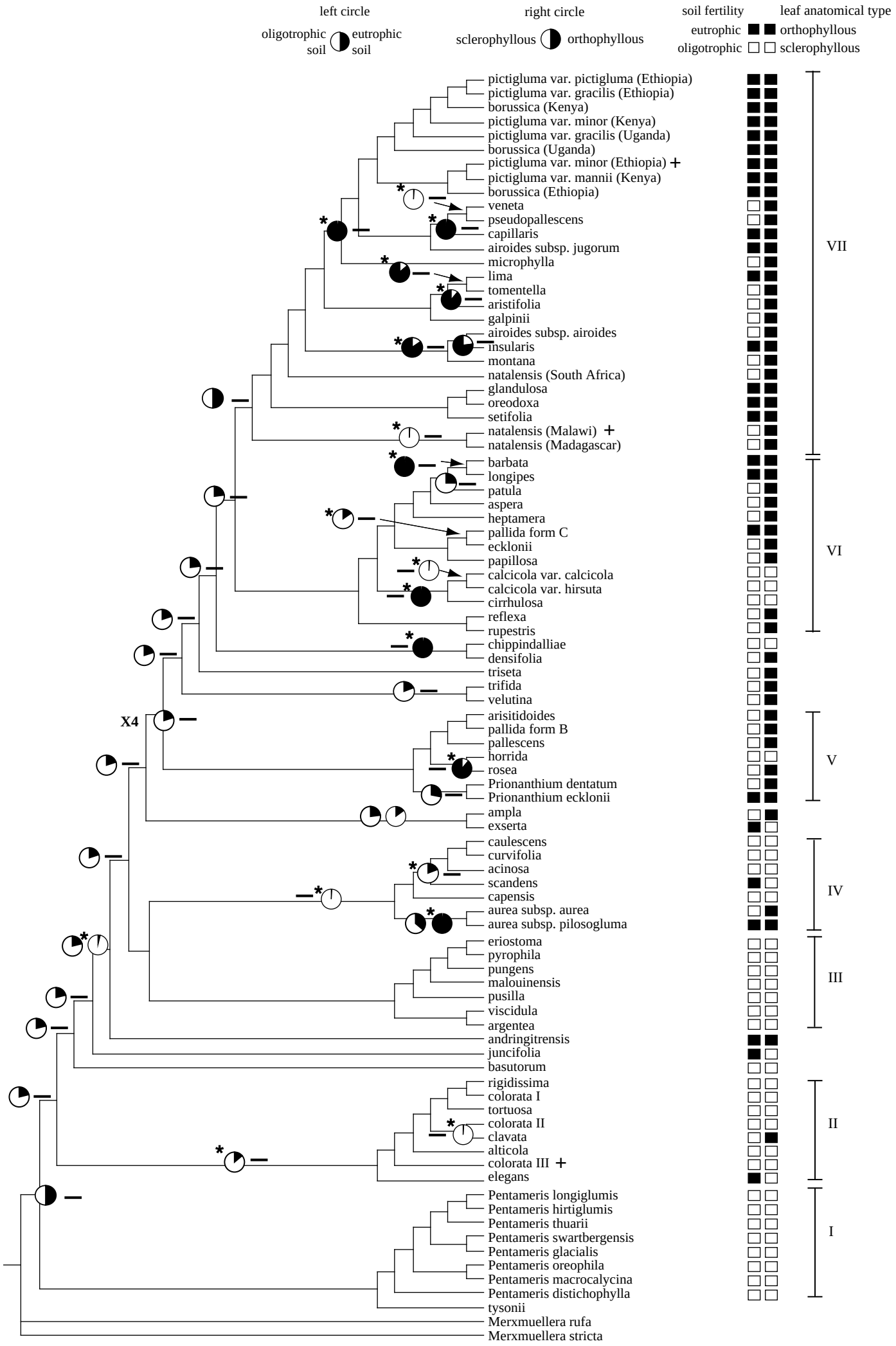
The earliest diverging members in the *Pentaschistis* clade tend to occur on oligotrophic soils. However node optimization for soil nutrient type was rarely decisive especially at the basal nodes (see Fig. 5), a phenomenon probably caused by the relatively low grouping information of the character (R.I. = 0.46 compared with glands R.I. = 0.65 and leaf anatomy type R.I. = 0.77). The difference in lnL considered as statistically significant was lowered to 1.4 (= 0.80 proportional likelihood) for optimization.

The balance of likelihood scores of character states along the 'spine' of the phylogeny shows the evolution of orthophyllous leaves from sclerophyllous leaves by node X4 (see Figs. 3 and 5), also change from oligotrophic to eutrophic soils (see Fig. 5). There is a strong bias in the occurrence of different leaf types on eutrophic and oligotrophic soils (see Table 5, in bold type; G-test $P < 0.000$, $df = 1$); sclerophyllous plants occur almost exclusively on oligotrophic soils, whereas orthophyllous plants occur more or less equally on both soil types. This can be decomposed to identify changes or maintenance of states at nodes, as shown in Table 5. Although sclerophyllous and orthophyllous taxa evolve onto eutrophic soils a more or less equal number of times, the bias between leaf anatomy type and soil nutrient type occurs because there is no diversification of sclerophyllous lineages on eutrophic soils (a conclusion that is upheld even if the complete phylogeny is considered). Conversely orthophyllous lineages diversify on both soil types, although the diversification rate (per change onto each soil type) is higher for eutrophic soils (~13 per change for eutrophic soils, vs. ~ six per change onto oligotrophic soils). Within the 'coastal clade' and the 'summer rainfall clade' especially, lineages change frequently between soil type and diversify on both.

Table 5. Number of 'events' of the two leaf anatomy types onto or within oligotrophic (0) soils or onto or within eutrophic (1) soils as well as pooled results for each soil type in bold.

		0 to 0	1 to 0	oligotrophic (total)	1 to 1	0 to 1	eutrophic (total)
leaf anatomy type	orthophyllous (0 to 0 and 1 to 0)	27	5	**32**	36	3	**39**
	sclerophyllous (1 to 1 and 0 to 1)	53	0	**53**	0	2	**2**

Figure 5. (following page) The left column of squares shows taxa as occurring on oligotrophic or eutrophic soils and the right column of squares shows taxa as sclerophyllous or orthophyllous. Arrows mark nodes that were not included in the analyses (see text). Unless otherwise illustrated, all nodes in clade X4 were optimized with statistical support as orthophyllous leaved (nodes outside this clade as sclerophyllous). Unless shown, all nodes in clade VI were optimized as eutrophic and nodes outside this clade as oligotrophic. Pie charts show node optimization (as proportional likelihoods) of nodes that are not ambiguously optimized, those subtending a change in leaf anatomy type, or other nodes of interest. Dashes are placeholders for pie charts that are not shown. Nodes are marked with asterisk when optimization is statistically significant (difference in lnL > 2). Clade names (I to VII) are informal names referred to in the text. Unless otherwise indicated all species are *Pentaschistis*.


left circle
oligotrophic soil
eutrophic soil
right circle
sclerophyllous
orthophyllous
soil fertility
eutrophic
oligotrophic
leaf anatomical type
orthophyllous
sclerophyllous
pictigluma var. pictigluma (Ethiopia)
pictigluma var. gracilis (Ethiopia)
borussica (Kenya)
pictigluma var. minor (Kenya)
pictigluma var. gracilis (Uganda)
borussica (Uganda)
pictigluma var. minor (Ethiopia) +
pictigluma var. mannii (Kenya)
borussica (Ethiopia)
veneta
pseudopallescens
capillaris
airoides subsp. jugorum
microphylla
lima
tomentella
aristifolia
galpinii
airoides subsp. airoides
insularis
montana
natalensis (South Africa)
glandulosa
oreodoxa
setifolia
natalensis (Malawi) +
natalensis (Madagascar)
barbata
longipes
patula
aspera
heptamera
pallida form C
ecklonii
papillosa
calcicola var. calcicola
calcicola var. hirsuta
cirrhulosa
reflexa
rupestris
chippindalliae
densifolia
triseta
trifida
velutina
X4
arisitidoides
pallida form B
pallescens
horrida
rosea
Prionanthium dentatum
Prionanthium ecklonii
ampla
exserta
caulescens
curvifolia
acinosa
scandens
capensis
aurea subsp. aurea
aurea subsp. pilosogluma
eriostoma
pyrophila
pungens
malouinensis
pusilla
viscidula
argentea
andringitrensis
juncifolia
basutorum
rigidissima
colorata I
tortuosa
colorata II
clavata
alticola
colorata III +
elegans
Pentameris longiglumis
Pentameris hirtiglumis
Pentameris thuarii
Pentameris swartbergensis
Pentameris glacialis
Pentameris oreophila
Pentameris macrocalycina
Pentameris distichophylla
tysonii
Merxmuellera rufa
Merxmuellera stricta
VII
VI
V
IV
III
II
I

DISCUSSION

We used DNA sequence data from four chloroplast regions and derived gap characters to reconstruct a phylogenetic hypothesis for the *Pentaschistis* clade. Both parsimony and Bayesian analysis provide moderately well-resolved and well-supported phylogenetic hypotheses with some exceptions, especially the spine of the tree (see Fig. 3). Analyses of individual datasets lacked well-supported resolution in this region, suggesting that lack of characters rather than conflict between datasets causes low resolution in these parts of the phylogeny. This is reflected in the very short branch lengths around these nodes in the individual analyses (data not shown).

We used a fully resolved phylogeny for ancestral character state reconstruction. We minimized the influence of branches which received less than 0.50 p.p. by giving them very short branch lengths. Also, in many cases nodes with low support could not be ambiguously optimized and were therefore omitted from further analysis. From inspection, there was only one region in the topology where different node arrangements would have altered the changes counted between character states, and the two nodes here were also omitted from the counts. Otherwise, most topological uncertainty was in terminal branches and most changes in soil type and gland losses especially, are fairly well dispersed throughout the phylogeny.

Systematics

The *Pentaschistis* clade was named as such following phylogenetic analysis of DNA sequence data of the Danthonioideae by Barker et al. (2000). The grouping of these three genera is also supported by morphological, histological and cytological features. These include the secondary loss or weak development of haustorial synergids, fine granular, or no starch in the synergids (compared to globular starch in other Danthonioid genera) (Verboom 1994), the insertion of the lemma setae in the sinuses between the lateral lobes and the median awn, although this is also found in *Pseudopentameris* (Verboom & Linder 1997), a basic chromosome number x = 7 (Spies & Roodt 2001) and palea veins that do not reach the tip of the palea.

The close relationship between *Pentaschistis* and *Pentameris* (Clayton & Renvoize 1986) has long been recognized. *Pentameris* was described by Palisot de Beauvois (1812) on account of four bristles (presumably two lemma lobes and two bristles) and the central awn of the lemma, also the shape and hairiness of the seed. The name *Pentaschistis* was first used 29

years later for a segregate of *Danthonia* (Nees ab Esenbeck 1841). The distinction between *Pentaschistis* and *Pentameris* has not always been clear and species were often transferred between the two genera (Kunth 1833; Nees ab Esenbeck 1841; Durand & Schinz 1895) or were considered in the same taxon (Durand and Schinz 1895). Stapf (1899) defined *Pentameris* based on the structure and hairiness of the ovary, recognising a total of five species. The two genera have since remained separated (e.g. McClean 1926; Barker 1993). This distinction is supported by our results, with the exception of *Pentaschistis tysonii* which is sister to *Pentameris*.

In contrast with *Pentameris* the close relationship between *Prionanthium* and *Pentaschistis* has only recently been recognized. When *Prionanthium* was first described, Desvaux (1831) noted that it was unlike any other known genus. Nees ab Esenbeck (1841) placed it in the tribe Phleoideae whereas other members of *Pentaschistis* were placed in the tribe Aveneae, in various genera (*Danthonia*, *Eriachne* and *Triraphis*). Similarly whereas the other members of the *Pentaschistis* clade were included in an expanded *Danthonia* D.C. by Durand and Schinz (1895), *Prionanthium* was included in *Phalaris*. Relationship of *Prionanthium* to the *Danthonia* group was first suggested by Chippindall (1955) and followed by Watson and Dallwitz (1992 onwards). Clayton and Renvoize (1986) include *Pentameris*, *Pentaschistis* and *Prionanthium* in the tribe Arundineae but regard *Prionanthium* as relatively distantly related to *Pentameris* and *Pentaschistis*, based on the short glumes and entire tipped lemmas of *Prionanthium* compared with the long glumes, bilobed lemmas and geniculate awns of the other two genera. Davidse (1988) pointed to four similarities in the spikelets of *Prionanthium* and *Pentaschistis*: the presence of multicellular glands (later supported by Linder et al. (1990) and Ellis (1989)), the two fertile florets per spikelet, the similarity in paleas and the rachilla extension above the upper floret. These results, based on morphological data are corroborated by our molecular data which show that *Prionanthium* is nested within *Pentaschistis*. The generic circumscription within the *Pentaschistis* clade as it currently stands does not reflect cladistic conventions and will be dealt with in a subsequent publication.

Erecting an infra-generic classification for *Pentaschistis* with its 70 species is fraught with difficulties. Currently there are two informal classifications available: the system of Linder and Ellis (1990) based on gland type and presence, leaf characteristics and spikelet size, and that of Ellis and Linder (1992) based on leaf anatomy. A formal infra-generic classification of *Pentaschistis* would be desirable to aid communication in this relatively large group. However, it would be difficult to recognize a reasonable number of monophyletic

subgenera or sections. Specifically, our phylogenetic tree contains five clades of six to 20 species, yet there are several single species or species pairs scattered throughout what is a rather pectinate tree. These isolated species and species pairs would necessitate up to seven further subgenera to be recognised. This topology, combined with the lack of obvious structural markers for these subgenera, would make the subsequent use of these subgenera and their identification in the field difficult. It additionally means that species not yet included in the molecular phylogenetic analysis cannot readily be placed. We therefore (regrettably) do not attempt to erect a sub-generic classification.

Evolution of Leaf Anatomy Type and the Presence of Glands

Both sclerophyllous and orthophyllous leaf anatomy types are found in different genera of the Danthonioideae (Ellis & Linder 1992), but *Pentaschistis* is unusual within the tribe as it includes both leaf anatomy types. We show that leaf anatomy type is a fairly labile character within the genus *Pentaschistis*, varying within well supported clades.

It is most probable that the multicellular glands evolved twice within the *Pentaschistis* clade, which is surprising given the otherwise rarity of these glands in the Poaceae. This multiple gain might be an artefact due to multiple extinctions of glanded species early on in the divergence of the *Pentaschistis* clade. While we cannot rule this out, the most likely hypothesis is that there are two origins. Alternatively, these gains may be the result of a 'latent homology' where the predisposition for a feature to evolve is a synapomorphy, not the structure itself (Stone & Hall 2004) (e.g. predisposition to nodulation within the nitrogen-fixing clade of Rosids I (Soltis et al. 1995); and probably eyes in animals (Fernald 2004)). It is also possible that *Pentaschistis clavata* is of hybrid origin, having inherited the glands from an unknown paternal parent and the chloroplast genome from *Pentaschistis colorata*.

Linder et al. (1990) hypothesised that the ancestral state in the clade was glandless and that linear glands were the first to evolve. From these, elongate sessile glands evolved and from these clavate glands. The sunken glands are proposed to be the most derived form of these gland types. This hypothesis was partially tested and supported but with low taxon sampling (Gilbert 2001). Our data support this general evolutionary pattern of increasing gland complexity within the clade, although glands may be lost from either type.

Glands evolved at, or near, the evolution of orthophyllous leaves. The rarity of these events precluded the use of the test of correlated evolution in Discrete (Pagel 1994), which

tests whether the transition rate in one (dependent) character is dependent upon the state of a second (independent) character. For our data the estimates of rate parameters were unrealistically high for both transitions that lead to a gain of glands (data not shown). Pagel (1994) suggested that for such values it is not possible to distinguish between different parameter values. This is probably caused by the rare evolution of glands. Consequently, we used ancestral character state reconstruction as the basis for an alternative approach to test these hypotheses.

Gained only twice, the multicellular glands have been lost at least 12 times, a pattern resembling that found in other complex structures. In the *Pentaschistis* clade some of the species are glandless because they are outside the clade in which glands evolved (the *Pentameris* clade (I), the *P. elegans* clade (II), *P. andringitrensis*, *P. basutorum* and *P. juncifolia*), but this does not explain all glandless taxa. Although glands have been lost against a background of both leaf types, if the opportunities for gland loss are taken into account, lineages with sclerophyllous taxa tend to lose glands much more often compared to orthophyllous leaved lineages.

It is also interesting to consider the number of diversification (= speciation minus extinction) events associated with different character combinations. Eglandular lineages with sclerophyllous leaves or glandular lineages with orthophyllous leaves represent the most 'stable' character combinations, and are associated with the most diversification events. In contrast lineages with sclerophyllous leaves and glands are associated with little lineage diversification, and lineages with neither sclerophyllous leaves nor glands arise several times (see Fig. 3) but none of these diversify. Whether this is due to a lower speciation rate or higher extinction rate is not clear, but these lineages may be 'evolutionary dead-ends'.

The loss of a structure may reflect selective forces different from those with which it originated (Gould 1997). However we propose a current function of the complex multicellular glands in the *Pentaschistis* clade by considering both character state correlations and the differences in the number of diversification events associated with different character state combinations. Specifically we hypothesise that sclerophyllous leaves and glands each act as a defence system against herbivory. Sclerophylly (Grubb 1986; Turner 1994), lignin (Moore and Jung 2001) and fibre content (Coley 1983; Grubb 1986; Turner 1994; Moore & Jung 2001) in leaves play a primary role in deterring herbivores. Likewise secretion from the glands of the *Pentaschistis* clade has a strong deterrent effect against insects in a choice-chamber experiment (Linder pers. obs.), although the active component of the exudates is unknown. The importance of such defence systems in this group is illustrated by the lineages

that lose both the glands and the sclerophyllous leaf type i.e. lose of both defence systems. Although these multiple gland losses are puzzling to explain, the absence of diversification of these lineages supports our hypothesis. This compares with losses of glands occurring alongside a transformation from orthophyllous to sclerophyllous leaf type. These represent an effective swap in the defence system and many of these lineages do diversify, retaining the sclerophyllous leaves as the defence system.

Given that there is a cost to producing and / or maintaining sclerophyllous leaves (Sobrado 1991) and glands, a cost / benefit scenario explains these observations and can be applied to the herbivory hypothesis outlined above (see Fig. 6). Having exclusively either defence system is the most 'stable' state, as the benefit in terms of defence against herbivory outweighs the cost. Having no defence system (orthophyllous leaves and no glands) is disadvantageous and lineages either fail to diversify, (re-)evolve either the glands or sclerophyllous leaves, or presumably go extinct. Conversely, if a plant has both systems redundancy means the benefit of the second system does not outweigh its cost, and this 'unstable' situation leads to the loss of one of the defence mechanisms.

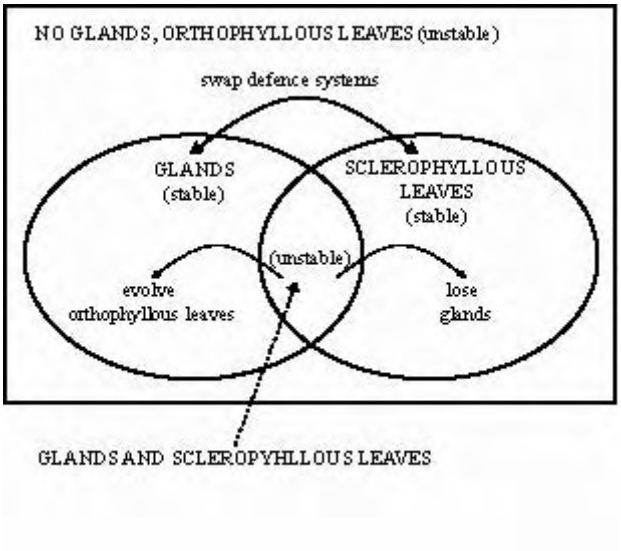


Figure 6 = Model of defence system hypothesis for the *Pentaschistis* clade.

In considering the diversification of lineages we combine several unknown factors, including the rates of diversification and extinction, as well as character state change. Although this means that we cannot estimate the rates of character state changes alone, we have the advantage of viewing the outcome of evolution. Specifically, we record the overall effect of the selective disadvantage / advantage of the different character combinations.

What remains to be understood is the 'drive' for such a change between the two alternative defence systems. To address this we tested the occurrence of taxa with different leaf types on soils with varying nutrient levels (eutrophic vs. oligotrophic). The CFR is well known for its mosaic of bedrock types, which give rise to both oligotrophic (notably

sandstone) and eutrophic soils. The bedrock variety on which the *Pentaschistis* clade grows is further varied due to its distribution on the more eutrophic basalts of the Drakensberg and tropical East African volcanoes. The lability of soil nutrient level across the phylogeny and the oversimplified partitioning of this continuous character into a binary character was probably the cause of the reduced statistical confidence in the node optimization.

Soil type does not explain the swap in defence systems from glands to sclerophyllous leaves, as lineages with orthophyllous leaf type (with glands) are just as likely to move onto oligotrophic soils as their sclerophyllous (glandless) relatives. There is however a significant bias between the two characters as diversification of sclerophyllous lineages is restricted to oligotrophic soils, whereas lineages of orthophyllous type evolve onto and diversify on both soil types. We suggest that the evolution of glands allowed orthophyllous taxa to persist and to diversify onto these more eutrophic soils, although they later revert to and diversify on oligotrophic soils.

Sclerophyllous leaves are associated with not only low nutrient level soils (especially P and N) (Beadle 1966; Cowling & Campbell 1983; Specht & Rundel 1990), but also water deficit (Schimper 1903; Connor & Doley 1981; Lamont *et al.* 2002). Across the CFR, where most of the *Pentaschistis* species occur, there is a strong rainfall seasonality gradient. One extreme is in the north-west (including Namaqualand), with its pronounced regime of winter rainfall and long summer drought, through to the eastern part of the CFR, with two rain seasons and no water deficit period (Schulze 1997). The occurrence of sclerophylly in the *Pentaschistis* clade however does not correlated with this. There are orthophyllous species found in Namaqualand and drier parts of the CFR, and sclerophyllous species found in the eastern part. Consequently it seems unlikely that sclerophylly is influenced strongly by water deficit.

Nutrient stress and water deficit however, are notoriously difficult to tease apart (Cunningham et al. 1999; Fonseca et al. 2000) and may have the same effects on the plant and / or even compound each other (Cunningham et al. 1999). Whether or not soil nutrient level was the primary factor in the evolution of the orthophyllous leaf type, it has played an important role in determining the differences in diversification between leaf anatomy types in the *Pentaschistis* clade, enabled by the evolution of glands.

ACKNOWLEDGEMENTS

We thank L. Andriamahefarivo, N. Bergh, B. Gehrke, Halima, Jaqueline, A. Verboom and staff at the BOL, PRE and MO herbaria for help during fieldwork and C. R. Hardy and T. van der Niet furthermore for many useful discussions. Thanks to A. Verboom and N. Barker for silica samples, and to M. Matthews for help with morphological preparations and for comments on the manuscript. We are also grateful for funding from the University of Zurich, the Swiss Academy of Sciences and the Georges und Antoine Claraz-Schenkung.

APPENDIX 1

Table 1. Collection and voucher data for DNA samples. * = material obtained from herbarium specimen

taxon	collection	collection locality and voucher location	trnLF	trnTL	atpB-rbcL	rpL16	trnCD
Merxmuellera rufa (Nees) Conert	T. van der Niet 11	Viljoenshof, Western Cape, South Africa (Z)	DQ913471	DQ913558	DQ913240		
Merxmuellera stricta (Schrad.) Conert	T. van der Niet 15	Uilkraal, Western Cape, South Africa (Z)	DQ913472	DQ913559	DQ913241	DQ913326	DQ913407
Pentameris distichophylla (Lehm.) Nees	A. Verboom 226	Hexrivier mountains, Western Cape, South Africa (BOL)	DQ913473	DQ913560	DQ913242	DQ913327	DQ913408
Pentameris glacialis N.P. Barker	H. P. Linder 5498	Great Swartberg, Western Cape, South Africa (BOL)	DQ913474	DQ913561	DQ913243		
Pentameris hirtiglumis N.P. Barker	H. P. Linder 7789	Hottentots Holland Nature Reserve, Western Cape, South Africa (Z)	DQ913475		DQ913244	DQ913328	DQ913409
Pentameris longiglumis (Nees) Stapf	C. Galley 536	Rockview Dam, Western Cape, South Africa (Z)	DQ913476	DQ913562	DQ913245	DQ913329	DQ913410
Pentameris macrocalycina (Steud.) Schweick.	A. Verboom 203	Cape Peninsula, Western Cape, South Africa (BOL)	DQ913477	DQ913563	DQ913246	DQ913330	DQ913411
Pentameris oreophila N.P. Barker	H. P. Linder 7802	Fonteintjiesberg, Western Cape, South Africa (Z)	DQ913478	DQ913564	DQ913247	DQ913331	DQ913412
Pentameris swartbergensis N.P. Barker	H. P. Linder 5490	Klein Swartberg, Western Cape, South Africa (BOL)	DQ913479	DQ913565	DQ913248		DQ913413
Pentameris thuarii Beauv.	H. P. Linder 5456	Montagu Pass, Western Cape, South Africa (BOL)	DQ913480	DQ913566	DQ913249	DQ913332	DQ913414
Pentaschistis acinosa Stapf	T. van der Niet 1	Landdroskop, Western Cape, South Africa (Z)	DQ913481	DQ913567	DQ913250	DQ913333	DQ913415
Pentaschistis airoides (Nees) Stapf subsp. *airoides*	H. P. Linder 6971	Kamiesberg, Northern Cape, South Africa (BOL)	DQ913482	DQ913568	DQ913251		DQ913416
Pentaschistis airoides (Nees) Stapf subsp. *Jugorum* (Stapf) H.P. Linder	C. Galley 81	Naudes Nek, Eastern Cape, South Africa (Z)	DQ913483	DQ913569	DQ913252	DQ913334	DQ913417
Pentaschistis alticola H.P. Linder	C. Galley 377	Ceres, Western Cape, South Africa (Z)		DQ913570	DQ913253	DQ913335	DQ913418
Pentaschistis ampla (Nees) McClean	A. Verboom 197	Paarl District, Western Cape, South Africa (BOL)	DQ913484	DQ913571	DQ913254	DQ913336	
Pentaschistis andringitrensis A.Camus	C. Galley 595	Massif d'Andringitra, Madagascar (Z)	DQ913485	DQ913572	DQ913255	DQ913337	DQ913419
Pentaschistis argentea Stapf	A. Verboom 254	Cape Peninsula, Western Cape, South Africa (BOL)	DQ913486	DQ913573	DQ913256	DQ913338	
Pentaschistis aristidoides (Thunb.) Stapf	T. van der Niet 37	Steenbergplateau, Western Cape, South Africa (Z)	DQ913487	DQ913574	DQ913257	DQ913339	
Pentaschistis aristifolia Schweick.	C. Galley 388	Clanwilliam, Western Cape, South Africa (Z)	DQ913488	DQ913575	DQ913258	DQ913340	DQ913420
Pentaschistis aspera (Thunb.) Stapf	A. Verboom 199	Cape Peninsula, Western Cape, South Africa (BOL)	DQ913489	DQ913576	DQ913259	DQ913341	
Pentaschistis aurea (Steud.) McClean subsp. *aurea*	H. P. Linder 6813	Katberg Pass, Western Cape, South Africa (BOL)	DQ913490	DQ913577	DQ913260		DQ913421
Pentaschistis aurea (Steud.) McClean subsp. *pilosogluma* (McClean) H.P. Linder	C. Galley 47	Monk's Cowl, Kwazulu-Natal, South Africa (Z)	DQ913491	DQ913578	DQ913261	DQ913342	DQ913422
Pentaschistis barbata (Nees) H.P. Linder	A. Verboom 219	Holbaai, Western Cape, South Africa (BOL)	DQ913492		DQ913262	DQ913343	
Pentaschistis basutorum Stapf	C. Galley 44	Golden Gate National Park, Free State, South Africa	DQ913493	DQ913579	DQ913263	DQ913344	DQ913423

		(Z)					
Pentaschistis borussica (K.Schum.) Pilg.	H. P. Linder 7661	Oromiya, Ethiopia (ETH)	DQ913494	DQ913580	DQ913264	DQ913345	DQ913424
Pentaschistis borussica (K.Schum.) Pilg.	C. Galley 230	Mount Elgon, Kenya (Z)	DQ913495	DQ913581	DQ913265	DQ913346	DQ913425
Pentaschistis borussica (K.Schum.) Pilg.	M. Namanganda 1353	Mt. Elgon, Uganda (Z)	DQ913496	DQ913582	DQ913266	DQ913347	DQ913426
Pentaschistis calcicola H.P. Linder var. *calcicola*	C. Galley 338	Bredasdorp, Western Cape, South Africa (Z)	DQ913497	DQ913583	DQ913267	DQ913348	DQ913427
Pentaschistis calcicola H.P. Linder var. *hirsuta* H.P. Linder	C. Galley 339	Bredasdorp, Western Cape, South Africa (Z)	DQ913498	DQ913584	DQ913268	DQ913349	DQ913428
Pentaschistis capensis (Nees) Stapf	H. P. Linder 6825	Bainskloof - Baviaanskloof, Western Cape, South Africa (BOL)	DQ913499	DQ913585	DQ913269	DQ913350	
Pentaschistis capillaris (Thunb.) McClean	C. Galley 322	Vredendal, Western Cape, South Africa (Z)	DQ913500	DQ913586	DQ913270	DQ913351	DQ913429
Pentaschistis caulescens H.P. Linder	C. Galley 376	Ceres, Western Cape, South Africa (Z)	DQ913501	DQ913587	DQ913271	DQ913352	DQ913430
Pentaschistis chippindalliae H.P. Linder	C. Galley 96	Long Tom's Pass, Mpumalanga, South Africa (Z)	DQ913502	DQ913588	DQ913272	DQ913353	DQ913431
Pentaschistis cirrhulosa (Nees) H.P. Linder	C. Galley 548	Stillbaai, Western Cape, South Africa (Z)	DQ913503	DQ913589	DQ913273	DQ913354	
Pentaschistis clavata Galley	C. Galley 567	Ceres, Western Cape, South Africa (Z)	DQ913504	DQ913590	DQ913274	DQ913355	DQ913432
Pentaschistis colorata (Steud.) Stapf	A. Verboom 213	Cape Peninsula, Western Cape, South Africa (BOL)	DQ913505	DQ913591	DQ913275	DQ913356	DQ913433
Pentaschistis colorata (Steud.) Stapf	C. Galley 343	Swellendam, Western Cape, South Africa (Z)	DQ913506	DQ913592	DQ913276		
Pentaschistis colorata (Steud.) Stapf	C. Galley 538	Gysmanshoek Pass, Western Cape, South Africa (Z)	DQ913507		DQ913277	DQ913357	DQ913434
Pentaschistis curvifolia (Schrad.) Stapf	T. van der Niet 53	Galgeberg, Western Cape, South Africa (Z)	DQ913508	DQ913593	DQ913278	DQ913358	DQ913435
Pentaschistis densifolia (Nees) Stapf	A. Verboom 225	Hexrivier mountains, Western Cape, South Africa (BOL)	DQ913509	DQ913594	DQ913279	DQ913359	DQ913436
* *Pentaschistis ecklonii* (Nees) McClean	H. P. Linder 6136	Malmesbury, Western Cape, South Africa (BOL)			DQ913280		
Pentaschistis elegans (Nees) Stapf	C. Galley 336	Bredasdorp, Western Cape, South Africa (Z)	DQ913510	DQ913595	DQ913281	DQ913360	DQ913437
Pentaschistis eriostoma (Nees) Stapf	H. P. Linder P6	Western Cape, South Africa (Z)	DQ913511	DQ913596	DQ913282	DQ913361	
Pentaschistis exserta H.P. Linder	C. Galley 51	Drakensberg, Kwazulu-Natal, South Africa (Z)	DQ913512	DQ913597	DQ913283	DQ913362	DQ913438
Pentaschistis galpinii (Stapf) McClean	C. Galley 42	Mount aux Sources, Kwazulu-Natal, South Africa (Z)	DQ913513	DQ913598	DQ913284	DQ913363	
Pentaschistis glandulosa (Schrad.) H.P. Linder	H. P. Linder 6814	Katberg Pass, Eastern Cape, South Africa (BOL)	DQ913514	DQ913599	DQ913285	DQ913364	DQ913439
Pentaschistis heptamera (Nees) Stapf	C. Galley 356	Uitenhague, Eastern Cape, South Africa (Z)	DQ913515	DQ913600	DQ913286	DQ913365	
Pentaschistis horrida Galley	T. van der Niet 20	Seweweekspoort, Western Cape, South Africa (Z)	DQ913516	DQ913601	DQ913287	DQ913366	DQ913440
* *Pentaschistis insularis* (Hemsl.) H.P. Linder	M. Lebouvier	BMG Junction, Amsterdam Island (private)	DQ913517				
Pentaschistis juncifolia Stapf	C. Galley 341	Swellendam, Western Cape, South Africa (Z)	DQ913518	DQ913602	DQ913288	DQ913367	DQ913441
Pentaschistis lima (Nees) Stapf	H. P. Linder 6972	Kamiesberg, Northern Cape, South Africa (BOL)	DQ913519	DQ913603	DQ913289	DQ913368	DQ913442
* *Pentaschistis longipes* Stapf	H. P. Linder 5018	Humansdorp, Western Cape, South Africa (BOL)			DQ913290		
Pentaschistis malouinensis (Steud.) Clayton	A. Verboom 218	Cape Peninsula, Western Cape, South Africa (BOL)	DQ913520	DQ913604	DQ913291	DQ913369	
Pentaschistis microphylla (Nees) McClean	C. Galley 76	Rhodes, Eastern Cape, South Africa (Z)	DQ913521	DQ913605	DQ913292	DQ913370	DQ913443

Pentaschistis montana H.P. Linder	C. Galley 574	Ceres, Western Cape, South Africa (Z)	DQ913522	DQ913606	DQ913293	DQ913371	
Pentaschistis natalensis (Stapf)	C. Galley 592	Ankaratra, Madagascar (Z)	DQ913523			DQ913372	
Pentaschistis natalensis (Stapf)	C. Galley 69	Mlunje Plateau, Malawi (Z)	DQ913524	DQ913607	DQ913294	DQ913373	DQ913444
Pentaschistis natalensis (Stapf)	C. Galley 95	Long Tom's Pass, Mpumalanga, South Africa (Z)	DQ913525	DQ913608	DQ913295	DQ913374	DQ913445
Pentaschistis oreodoxa (Schweick.)	C. Galley 32	Mount aux Sources, Kwazulu-Natal, South Africa (Z)	DQ913526	DQ913609	DQ913296	DQ913375	DQ913446
Pentaschistis pallescens (Schrad.) Stapf	A. Verboom 216	Cape Peninsula, Western Cape, South Africa (BOL)	DQ913527	DQ913610	DQ913297	DQ913376	DQ913447
Pentaschistis pallida (Thunb.) H.P. Linder form B	T. van der Niet 32	Kogelberg, Western Cape, South Africa (Z)	DQ913528	DQ913611	DQ913298	DQ913377	
Pentaschistis pallida (Thunb.) H.P. Linder form C	C. Galley 547	Stillbaai, Western Cape, South Africa (Z)	DQ913529	DQ913612		DQ913378	
Pentaschistis papillosa (Steud.) H.P. Linder	A. Verboom 209	Silvermine Nature Reserve, Western Cape, South Africa (BOL)	DQ913530	DQ913613		DQ913379	
Pentaschistis patula (Nees) Stapf	C. Galley 317	Clanwilliam, Western Cape, South Africa (Z)	DQ913531	DQ913614	DQ913299	DQ913380	DQ913448
Pentaschistis pictigluma (Steud.) Pilger var. *gracilis* (S.M.Phillips) S.M. Phillips	H. P. Linder 7676	Oromiya, Ethiopia (ETH)	DQ913532	DQ913615	DQ913300	DQ913381	DQ913449
Pentaschistis pictigluma (Steud.) Pilger var. *gracilis* (S.M.Phillips) S.M. Phillips	M. Namanganda 1358	Mt. Elgon, Uganda (Z)	DQ913533		DQ913301	DQ913382	DQ913450
Pentaschistis pictigluma (Steud.) Pilger var. *mannii* S.M. Phillips	C. Galley 267	Mount Kenya, Kenya (Z)	DQ913534	DQ913616	DQ913302	DQ913383	DQ913451
Pentaschistis pictigluma (Steud.) Pilger var. *minor* S.M. Phillips	H. P. Linder 7671	Bale Mountains, Ethiopia (ETH)	DQ913535	DQ913617	DQ913303	DQ913384	
Pentaschistis pictigluma (Steud.) Pilger var. *minor* S.M. Phillips	C. Galley 270	Mount Kenya, Kenya (Z)	DQ913536	DQ913618	DQ913304	DQ913385	DQ913452
Pentaschistis pictigluma (Steud.) Pilger var. *pictigluma* S.M. Phillips	H. P. Linder 7670	Bale Mountains, Ethiopia (ETH)	DQ913537	DQ913619	DQ913305	DQ913386	DQ913453
Pentaschistis pseudopallescens H.P. Linder	C. Galley 379	Hexrivier mountains, Western Cape, South Africa (Z)	DQ913538	DQ913620	DQ913306	DQ913387	DQ913454
Pentaschistis pungens H.P. Linder	C. Galley 333	Clanwilliam, Western Cape, South Africa (Z)	DQ913539	DQ913621	DQ913307	DQ913388	DQ913455
Pentaschistis pusilla (Nees) H.P. Linder	A. Verboom 206	Cape Peninsula, Western Cape, South Africa (BOL)	DQ913540	DQ913622	DQ913308	DQ913389	DQ913456
Pentaschistis pyrophila H.P. Linder	A. Verboom 229	Hexrivier mountains, Western Cape, South Africa (BOL)	DQ913541	DQ913623	DQ913309	DQ913390	
Pentaschistis reflexa H.P. Linder	C. Galley 324	Clanwilliam, Western Cape, South Africa (Z)	DQ913542	DQ913624	DQ913310	DQ913391	DQ913457
Pentaschistis rigidissima Pilger ex H.P. Linder	A. Verboom 227	Hexrivier mountains, Western Cape, South Africa (BOL)	DQ913543	DQ913625	DQ913311	DQ913392	DQ913458
Pentaschistis rosea H.P. Linder subsp. *purpurascens* H.P. Linder	C. Galley 378	Hexrivier mountains, Western Cape, South Africa (Z)	DQ913544	DQ913626	DQ913312	DQ913393	DQ913459
Pentaschistis rupestris (Nees) Stapf	A. Verboom 251	Hexrivier mountains, Western Cape, South Africa (BOL)	DQ913545	DQ913627	DQ913313	DQ913394	DQ913460
Pentaschistis scandens H.P. Linder	C. Galley 334	Bredasdorp, Western Cape, South Africa (Z)	DQ913546	DQ913628	DQ913314	DQ913395	
Pentaschistis setifolia (Thunb.) McClean	C. Galley 45	Golden Gate National Park, Free State, South Africa (Z)	DQ913547	DQ913629	DQ913315	DQ913396	DQ913461

Pentaschistis tomentella Stapf	C. Galley 318	Calvinia, Northern Cape, South Africa (Z)	DQ913548	DQ913630	DQ913316	DQ913397	DQ913462
Pentaschistis tortuosa (Trin.) Stapf	A. Verboom 250	Hexrivier mountains, Western Cape, South Africa (BOL)	DQ913549	DQ913631	DQ913317	DQ913398	DQ913463
Pentaschistis trifida Galley	C. Galley 577	Ceres, Western Cape, South Africa (Z)	DQ913550	DQ913632	DQ913318	DQ913399	DQ913464
Pentaschistis triseta (Thunb.) Stapf	H. P. Linder 6962	Romansrivier, Western Cape, South Africa (BOL)	DQ913551	DQ913633	DQ913319	DQ913400	DQ913465
Pentaschistis tysonii Stapf	H. P. Linder 6812	Katberg Pass, Western Cape, South Africa (BOL)	DQ913552	DQ913634	DQ913320	DQ913401	DQ913466
Pentaschistis velutina H.P. Linder	C. Galley 389	Clanwilliam, Western Cape, South Africa (Z)	DQ913553	DQ913635	DQ913321	DQ913402	DQ913467
Pentaschistis veneta H.P. Linder	C. Galley 576	Ceres, Western Cape, South Africa (Z)	DQ913554	DQ913636	DQ913322	DQ913403	DQ913468
Pentaschistis viscidula (Nees) Stapf	H. P. Linder 7787	Hottentots Holland Nature Reserve, Western Cape, South Africa (Z)	DQ913555	DQ913637	DQ913323	DQ913404	
Prionanthium dentatum (L.f.) Henrard	H. P. Linder 5430	Nieuwoudtville, Western Cape, South Africa (BOL)	DQ913556	DQ913638	DQ913324	DQ913405	DQ913469
Prionanthium ecklonii (Nees) Stapf	H. P. Linder 5402	Clanwilliam, Western Cape, South Africa (BOL)	DQ913557	DQ913639	DQ913325	DQ913406	DQ913470

REFERENCES CITED

Akaike, H. 1973 Information theory and an extension of the maximum likelihood principle. In *Proceedings of the second international symposium in information theory* (ed. B. N. Petrov & F. Csaki), pp. 267-281. Budapest: Akademiai Kiado.

APG. 2003 An update of the Angiosperm Phylogeny Group classification for the orders of families of flowering plants: APG II. *Bot. J. Linn. Soc.* **141**, 399-436.

Armbruster, W. S. 1992 Phylogeny and the evolution of plant-animal interactions. *Biosciences* **42**, 12-20.

Barker, N. P. 1993 A biosystematic study of *Pentameris* (Arundineae, Poaceae). *Bothalia* **23**, 25-47.

Barker, N. P., Morton, C. M. & Linder, H. P. 2000 The Danthonieae: generic composition and relationships. In *Grasses: systematics and evolution* (ed. J. Everett), pp. 221-230. Melbourne: CSIRO.

Baum, D. A., Small, R. L. & Wendel, J. F. 1998 Biogeography and floral evolution of baobabs (*Adansonia*, Bombacaceae) as inferred from multiple data sets. *Syst. Biol.* **47**, 181-207.

Beadle, N. C. W. 1966 Soil phosphate and its role in molding segments of the Australian flora and vegetation, with special reference to xeromorphy and sclerophylly. *Ecology* **47**, 992-1007.

Bungard, R. A. 2004 Photosynthetic evolution in parasitic plants: insight from the chloroplast genome. *Bioessays* **26**, 235-247.

Burnham, K. P. & Anderson, D. R. 2004 Multimodel inference, understanding AIC and BIC in model selection. *SMR* **33**, 261-304.

Chippindall, L. K. A. 1955 A guide to the identification of grasses in South Africa. In *The grasses and pastures of South Africa* (ed. D. Meredith). Johannesburg: Central News Agency.

Clayton, W. D. & Renvoize, S. A. 1986 *Genera Graminum*. Kew Bulletin Additional Series. London: Royal Botanical Garden Kew.

Coddington, J. A. 1988 Cladistic tests of adaptationaly hypotheses. *Cladistics* **4**, 3-22.

Coley, P. D. 1983 Herbivory and defensive characteristics of tree species in a lowland tropical forest. *Ecol. Monogr.* **53**, 209-233.

Connor, D. J. & Doley, D. 1981 The water relations of heathlands: physiological adaptation to drought. In *Heathlands and Related Shrublands*, vol. 9B (ed. R. L. Specht), pp. 131-141. Amsterdam, Oxford, New York: Elsevier.

Cowling, R. M. & Campbell, B. M. 1983 The definition of leaf consistence categories in the fynbos biome and their distribution along an altitudinal gradient in the south eastern Cape. *J. S. African Bot.* **49**, 87-101.

Cunningham, S. A., Summerhayes, B. & Westoby, M. 1999 Evolutionary divergences in leaf structure and chemistry, comparing rainfall and soil nutrient gradients. *Ecology* **69**, 569-588.

Darwin, C. 1862 *On the various contrivances by which British and foreign orchids are fertilised by insects*. London: John Murray.

Davidse, D. 1988 A revision of the genus *Prionanthium* (Poaceae : Arunidnoideae). *Bothalia* **18**, 143-153.

Dawkins, R. 1986 *The blind watchmaker*. England: Penguin Books Limited.

Desvaux, A.-N. 1831 *Opuscules sur les sciences physiques et naturelles*. Angers: Pavie.

Doyle, J. J. & Doyle, J. L. 1987 A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochem. Bull.* **19**, 11-15.

du Plessis, H. & Spies, J. J. 1992 Chromosome numbers in the genus *Pentaschistis* (Poaceae, Danthonioideae). *Taxon* **41**, 709-720.

Durand, T. & Schinz, H. 1895 *Conspectus florae africae*. Paris: Klincksieck.

Ellis, R. P. 1989 Leaf anatomy of the South African Danthonieae (Poaceae): XIX. The genus *Prionanthium*. *Bothalia* **19**, 217-223.
Ellis, R. P. & Linder, H. P. 1992 Atlas of the leaf anatomy in *Pentaschistis* (Arundineae: Poaceae). *Mem. Bot. Surv. South Africa* **60**.
Felsenstein, J. 1985a Confidence limits on phylogenies: and approach using the bootstrap. *Evolution* **39**, 783-791.
Felsenstein, J. 1985b Phylogenies and the comparative method. *The American Naturalist* **125**, 1-15.
Fernald, R. D. 2004 Evolving eyes. *Int. J. Dev. Biol.* **48**, 701-705.
Fonseca, C. R., Overton, J. M., Collins, B. & Westoby, M. 2000 Shifts in trait-combinations along rainfall and phosphorus gradients. *J. Ecol.* **88**, 964-977.
Galley, C. & Linder, H. P. 2006 New species and taxanomic changes within *Pentaschistis* (Danthonioideae, Poaceae) from the Western Cape Province, South Africa. *Bothalia* **36**, 157-162.
Gilbert, M. E. 2001 The *Pentaschistis* clade: anatomical and morphological evolution. In *Department of Botany*. Grahamstown: Rhodes University.
Goldblatt, P. 1978 An analysis of the flora of Southern Africa: its characteristics, relationships, and origins. *Annals of the Missouri Botanical Garden* **65**, 369-436.
Gould, S. J. 1997 The exaptive excellence of spandrels as a term and prototype. *Proc. Natl. Acad. Sci. USA* **94**, 10750-10755.
Grubb, P. J. 1986 Sclerophylls, pachyphylls and pycnophylls: the nature and significance of hard leaf surfaces. In *Insects and plant surfaces* (ed. B. E. Juniper & T. R. E. Southwood), pp. 137-150. London: Edward Arnold Publishers Ltd.
Huelsenbeck, J. P. & Ronquist, F. 2001 MRBAYES: Bayesian inference of phylogenetic trees. *Bioinformatics* **17**, 754-755.
Igersheim, A. & Cichock, O. 1996 A simple method for microtome sectioning of prehistoric charcoal specimens, embedded in 2-hydroxy-ethyl methacrylate (HEMA). *Rev. Palaeobot. Palynol.* **92**, 389-393.
Judd, W. S., Campbell, C. S., Kellogg, E. A., Stevens, P. F. & Donoghue, M. J. 2002 *Plant systematics, a phylogenetic approach*. Sunderland, Massachusetts, USA: Sinauer Associates, Inc.
Killick, D. J. B. 1979 African mountain heathlands. In *Heathlands and Related Shrublands*, vol. 9A (ed. R. L. Specht), pp. 97-115. Amsterdam, Oxford, New York: Elsevier.
Knapp, R. 1973 *Die vegetation von Afrika*. Jena: Veb Gustav Fischer-Verlag.
Kohn, J. R., Graham, S. W., Morton, B., Doyle, J. J. & Barrett, S. C. H. 1996 Reconstruction of the evolution of reproductive characters in Pontederiaceae using phylogenetic evidence from chloroplast DNA restriction-site variation. *Evolution* **50**, 1454-1469.
Kruger, F. J. 1979 South African Heathlands. In *Heathlands and Related Shrublands*, vol. 9A (ed. R. L. Specht), pp. 19-79. Amsterdam, Oxford, New York: Elsevier.
Kunth, C. S. 1833 *Enumeratio plantarum I.* Stuttgart: Cotta.
Lambrechts, J. J. N. 1979 Geology, geomorphology and soils. In *Fynbos ecology: a preliminary synthesis* (ed. J. Day, W. R. Siegfried, G. N. Louw & M. L. Jarman), pp. 16-26. Pretoria: Souther African National Scientific Programmes Report no. 40.
Lamont, B. B., Groom, P. K. & Cowling, R. M. 2002 High leaf mass per area of related species assemblages may reflect low rainfall and carbon isotope discrimination rather than low phosphorus and nitrogen concentrations. *Funct. Ecol.* **16**, 403-412.
Linder, H. P. 1989 Grasses in the Cape Floristic Region: Phytogeographical implications. *S. African J. Sci.* **85**, 502-505.
Linder, H. P. & Ellis, R. P. 1990 A revision of *Pentaschistis* (Arundineae: Poaceae). *Contr. Bolus Herb.* **12**.

Linder, H. P., Thompson, J. F., Ellis, R. P. & Perold, S. M. 1990 The occurance, anatomy, and systematic implications of the glands in *Pentaschistis* and *Prionanthium* (Poaceae, Arundinoideae, Arundineae). *Bot. Gaz.* **151**, 221-233.
Luckow, M. & Bruneau, A. 1997 Circularity and independence in phylogenetic tests of ecological hypotheses. *Cladistics* **13**, 145-151.
Maddison, W. P. & Maddison, D. R. 2006
McClean, A. P. D. 1926 The history, phylogeny and taxonomy of the genus *Achneria* Munro. *S. African J. Sci.* **23**, 273-282.
Mooers, A. O. & Schluter, D. 1999 Reconstructing ancestral states with maximum likelihood: support for one- and two-rate models. *Syst. Biol.* **48**, 623-633.
Moore, K. J. & Jung, H. G. 2001 Lignin and fiber digestion. *J. Range Managem.* **54**, 420-430.
Nees ab Esenbeck, C. G. 1841 *Florae africanae australioris I. Gramineae*. Glogau: Prausnitzianis.
Nickrent, D. L., Blarer, A., Qui, Y.-L., Vidal-Russell, R. & Anderson, F. E. 2004 Phylogenetic inference in Rafflesiales: the influence of rate heterogeneity and horizontal gene transfer. *BMC Evolutionary Biology* **4**, Art. No. 40.
Nixon, K. C. 1999-2002 The Author, *1.0000*.
Nylander, J. A. A., Ronquist, F., Huelsenbeck, J. P. & Nieves-Aldrey, J. L. 2004 Bayesian phylogenetic analysis of combined data. *Syst. Biol.* **53**, 47-67.
Pagel, M. 1994 Detecting correlated evolution of phylogenies: a general method for comparative analysis of discrete characters. *Proc. R. Soc. Lond. B.* **225**, 37-45.
Pagel, M. 1999 The maximum likelihood approach to reconstructing ancestral character states of discrete characters on phylogenies. *Syst. Biol.* **48**, 612-622.
Pagel, M. & Harvey, P. H. 1988 Recent developments in the analysis of comparative data. *Quart. Rev. Biol.* **63**, 413-440.
Palisot de Beauvois, A. M. F. J. 1812 *Essai d'une Nouvelle Agrostographie*. Paris: Fain.
Phillips, S. M. 1994 Variation in the *Pentaschistis pictigluma* complex (Gramineae). In *XIIIth Plenary Meeting AETFAT, Malawi*, vol. 1 (ed. J. H. Seyani & A. C. Chikuni), pp. 359-372. Zomba, Malawi: National Herbarium and Botanic Gardens of Malawi.
Phillips, S. M. 1995 A new species of *Pentaschistis* (Gramineae) from Ethiopia. *Kew Bull.* **50**, 615-617.
Posada, D. & Crandall, K. A. 1998 Modeltest: testing the model of DNA substitution. *Bioinformatics* **14**, 817-818.
Sanderson, M. J. 2002 Estimating absolute rates of molecular evolution and divergence times: A penalized likelihood approach. *Molec. Biol. Evol.* **19**, 101-109.
Sanderson, M. J. 2003 r8s; inferring absolute rates of evolution and divergence times in the absence of a molecular clock. *Bioinformatics* **9**, 301-302.
Schimper, A. F. W. 1903 *Plant Geographic Upon a Physiological Basis*. Oxford, UK: Clarendon Press.
Schluter, D., Price, T., Mooers, A. O. & Ludwig, D. 1997 Likelihood of ancestor states in adaptive radiation. *Evolution* **51**, 1699-1711.
Schoen, D. J., Johnston, M. O., l'Heureux, A.-M. & Marsolais, J. V. 1997 Evolutionary history of the mating system in *Amsinckia* (Boraginaceae). *Evolution* **51**, 1090-1099.
Schulze, R. E. 1997 South African Atlas of Agrohydrology and Climatology, Report TT82/96. Pretoria: Water Research Commission.
Sculthorpe, C. D. 1967 *The biology of aquatic vascular plants*. London: Edward Arnold Publishers Ltd.
Shaw, J., Lickey, E. B., Beck, J. T., Farmer, S. B., Lui, W., Miller, J., Siripun, K. C., Winder, C. T., Schilling, E. E. & Small, R. L. 2005 The tortoise and the hare II: Relative utility of 21 noncoding chloroplast DNA sequences for phylogenetic analyses. *Amer. J. Bot.* **92**, 142-166.

Sillén-Tullberg, B. 1993 The effect of biased inclusion of taxa on the correlation between discrete characters in phylogenetic trees. *Evolution* **47**, 1182-1191.
Simmons, M. P. & Ochoterena, H. 2000 Gaps as characters is sequence-based phylogenetic analyses. *Syst. Biol.* **49**, 369-381.
Sobrado, M. A. 1991 Cost-benefit relationships in deciduous and evergreen leaves of tropical dry forest species. *Functional Ecology* **5**, 608-616.
Sokal, R. R. & Rohlf, F. J. 1995 *Biometry*. New York: Freeman.
Soltis, D. E., Soltis, P. S., Morgan, D. R., Swensen, S. M., Mullin, B. C., Dowd, J. M. & Martin, P. G. 1995 Chloroplast gene sequence data suggest a single origin of the predisposition for symbiotic nitrogen fixation in angiosperms. *Proc. Natl. Acad. Sci. USA* **92**, 2647-2651.
Specht, R. L. & Rundel, P. W. 1990 Sclerophylly and foliar nutrient status of Mediterranean-climate plant communities in southern Australia. *Austral. J. Bot.* **38**.
Spies, J. J. & Roodt, R. 2001 The basic chromosome number of the genus *Pentameris* (Arundinoideae). *Bothalia* **31**, 145-146.
Stapf, O. 1899 Volume VII Gramineae. In *Flora Capensis* (ed. W. T. Thiselton-Dyer), pp. 1-791. London: Lovell Reeve and Co.
Stone, J. R. & Hall, B. K. 2004 Latent homologues for the neural crest as an evolutionary novelty. *Evol. & Developm.* **6**, 123-129.
Swofford, D. L. 2002
Taberlet, P., Gielly, L., Pautou, G. & Bouvet, J. 1991 Universal primers for amplification of three non-coding regions of chloroplast DNA. *Plant Molec. Biol.* **17**, 1105-1109.
Tolivia, D. & Tolivia, J. 1987 Fasga - a new polychomatic method for simultaneous and differential staining of plant tissues. *J. Microscopy* **148**, 113-117.
Turner, I. M. 1994 Sclerophylly: primarily protective? *Funct. Ecol.* **8**, 669-675.
Verboom, G. A. 1994 Haustorial synergids: An important character in the systematics of Danthonioid grasses (Arundinoideae: Poaceae)? *Amer. J. Bot.* **81**, 1601-1610.
Verboom, G. A. & Linder, H. P. 1997 A re-evaluation of species limits in *Chaetobromus* (Danthonioideae: Poaceae). *Nordic J. Bot.* **18**, 57-78.
Watson, L. & Dallwitz, M. J. 1992 onwards The grass genera of the world: descriptions, illustrations, identification, and information retrieval; including synonyms, morphology, anatomy, physiology, phytochemistry, cytology, classification, pathogens, world and local distribution, and references. http://delta-intkey.com.
Werdelin, L. & Tullberg, B. S. 1995 A comparison of two methods to study correlated discrete characters on phylogenetic trees. *Cladistics* **11**, 265-277.
Whiting, M. F., Bradler, S. & Maxwell, T. 2003 Loss and recovery of wings in stick insects. *Nature* **421**, 264-267.
Witkowski, E. T. F. & Mitchell, D. T. 1987 Variations in soil phosphorus in the fynbos biome, South Africa. *J. Ecol.* **75**, 1159-1171.

How do so many *Pentaschistis* species fit into the Cape region, South Africa?

CHLOE GALLEY AND H. PETER LINDER

Institute of Systematic Botany, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland

Diversity and Distributions: in prep

This chapter was co-supervised by Niklaus Zimmermann and in the final paper he will be included as a co-author (Swiss Federal Institute for Forest, Snow and Landscape Research, Zürcherstrasse 111, Birmensdorf, Switzerland).

ABSTRACT

We investigate the role of habitat heterogeneity in allowing so many species of the *Pentaschistis* clade to occur in the Cape Floristic Region (South Africa). This region is in general very species rich for both its area and latitudinal position. There are 65 (sub-)species of the *Pentaschistis* clade in the Cape Floristic Region and 11 species in the Drakensberg, and this provides a contrasting area whereby age and lineage-specific effects can be excluded. A combination of environmental variables are used, and categorised to construct units of habitat which are used as comparative macrohabitat units between the regions and between the species. The amount of habitat available in the Cape and Drakensberg is not significantly different, but the amount of habitat occupied by *Pentaschistis* in the Cape is significantly higher than in the Drakensberg. Estimated niche size of the species in each region is similar, but more species can fit in one habitat unit in the Cape, and we suggest that microhabitat niches play an important role in allowing coexistence in a landscape. Higher species turnover in the Cape compared to the Drakensberg is not explained by a greater turnover in habitats. Both extreme and partially range restricted species need to be explained to understand species turnover. The range restricted species in *Pentaschistis* do not have characteristics of typical range restricted species of the Cape. We show that some species are range restricted because they occupy range restricted niches, whereas others have widespread niches but do not occupy the full geographical range of these.

Let's consider the concept of niche –

If I knew what it meant I'd be rich.
Its dimensions are n
But a knowledge of Zen
Is required to fathom the bitch

- Grant Cottam and David Parkhust

INTRODUCTION

Species richness is not evenly distributed around the world. The richness of different regions at a large spatial scale is commonly correlated with area and (Rosenzweig 1995) latitude (Rosenzweig 1995, and references therein; Mutke & Barthlott 2005). The Cape Floristic Region (Goldblatt 1978, hereafter referred to as the 'Cape') in southern Africa breaks both of these rules; it is unusually species rich for its area and latitude, comparable only to mega species rich tropical rainforests (Linder 2003). A number of hypotheses invoking historical and / or contemporary aspects have been proposed to explain the high species richness of the Cape; most notably, hypotheses relating to the complex patterns of soil types (Goldblatt & Manning 2002), the rainfall quantity or range (Linder 1991; Goldblatt & Manning 2002), complex fire regimes (Cowling 1987), or the variation in pollinator systems (Johnson & Steiner 2003); also, Pliocene and / or Pleistocene climatic stability reducing extinction rate (Cowling 1983; Cowling & Lombard 2002; Goldblatt & Manning 2002) and the combined effects of limited dispersal and high speciation rate (Cowling & Holmes 1992; Simmons & Cowling 1996; Latimer et al. 2005). However there seems to be no consensus regarding which is the most important factor or set of factors (Linder 2003; Savolainen & Forest 2005).

Species richness of a region is partitioned into several levels, and also includes both richness and turnover (Whittaker 1977; Whittaker *et al.* 2001). The alpha diversity of the Cape flora is substantial compared to other Mediterranean floras (Cowling *et al.* 1996), but not outstanding on a regional or world scale (Cowling 1990; Cowling *et al.* 1992). Species turnover along habitat gradients (beta diversity) is fairly high (Linder 1985; Cowling 1990; Cowling et al. 1992). Along geographical gradients the turnover also very high (Cowling 1990), possibly unmatched anywhere else in the world (Kruger & Taylor 1979). Similarly, the species richness within a landscape is comparable only with species rich East Asian tropical rainforests (Kruger & Taylor 1979). Dissecting apart the species richness of an area to

distinguish these different levels enables us to better understand the processes which lead to the species diversity we see today (Cody 1986; Ricklefs 2006).

Identifying which factors might be important in allowing species richness to persist requires a comparative area. Many plant taxa that are species-rich in the Cape also occur in the Drakensberg Region, where they tend to be relatively species poor (Hilliard & Burtt 1987; Carbutt & Edwards 2002). The Drakensberg therefore presents a good comparison with which to investigate the species richness in the Cape. One such taxon is the *Pentaschistis* clade (*Pentameris*, *Pentaschistis*, *Prionanthium*; hereafter referred to as '*Pentaschistis*'), with 65 species in the Cape and 11 species in the Drakensberg. It is a common element in the grasslands in the Drakensberg and the fynbos vegetation in the Cape, and is the most species rich grass clade of the Cape flora. The taxonomy of the *Pentaschistis* clade is relatively well known (Davidse 1988; Linder & Ellis 1990; Barker 1993; Phillips 1994; Phillips 1995; Galley & Linder 2006b), allowing us to use comparable taxonomic units.

Using a taxon which is widespread across two regions has the advantage that we can check for phylogenetic relatedness, and for differences in the ages of species between the two regions. If these factors can be excluded, the argument remaining is that either increased speciation rate and / or increased persistence are important. Part of persistence is related to the availability of habitats (Szabo & Meszéna 2006) and we test the role of habitat heterogeneity in explaining the higher species richness in the Cape compared to the Drakensberg. Both landscape richness and turnover between landscapes are investigated.

METHODS

Sampling

We obtained 2158 locality records for 63 of the 65 species in the Cape, and 359 locality records for 11 species in the Drakensberg region, from the Bolus Herbarium (University of Cape Town) and the National Herbarium (South African National Biodiversity Institute, Pretoria). We followed the taxonomy of Linder and Ellis (1990), Barker (1993) and Davidse (1988) and included recently described species (Galley & Linder 2006b). Sub-species were considered separate from one another but other infra-specific taxa were combined. *Pentaschistis pallida* is a species complex of seven forms which often created outlying data points for variables tested; therefore we excluded it from the analyses. All localities were digitised to a 1 x 1 minute resolution, or used as given (for GPS localities).

Environmental data

The rasters datasets of climatic data for Southern Africa were obtained from the Schulze Atlas (Schulze 1997), see Table 1. Additionally a 'continentality' dataset was derived from the mean maximum daily temperatures in January minus the mean minimum daily temperatures in July. The cell size of all layers corresponds to ~1.6 x 1.4 km^2. All data preparation and analyses were carried out in ArcGIS (1999-2002).

The raster datasets were transformed into shape files and spatial joins were used to attach the climatic data to each plant record. This dataset was split into taxa which are centred in the two regions: Cape or the Drakensberg. Due to analytical constraints only five environmental variables could be used to represent 'bioclimatic units', from which the niche characteristics of the species and the niches available in the landscape were estimated. A discriminant function (DF) analysis was therefore used to show which variables are important in separating the species within the two regions (see Appendix 1, Table 1a, b). Secondly, data were standardised and a correlation matrix was calculated to identify highly correlated variables (see Appendix 1, Table 2a, b). Five variables were chosen which both contributed substantially to the first four axes in the DF analysis and which were least correlated with each other. These were potential evaporation (APAN), continentality, degree days in the summer, frost days and mean annual precipitation (MAP) (see table 1). Bedrock type was also investigated as a potential variable. Being an unordered character it could not be tested using the DF analysis but as many *Pentaschistis* species are restricted to soils from one or two bedrock types, and bedrock type has been included in many hypotheses of explaining the species richness of the Cape (Oliver et al. 1983; Cowling 1990; Cowling et al. 1992; Simmons & Cowling 1996; Goldblatt & Manning 2002), it is potentially important. Bedrock type replaced 'degree days in the summer' (which performed least well out of the five variables). However, the predictive modelling behaved very poorly with bedrock compared with a purely climatic dataset and we therefore performed the rest of the analyses using only the set of climatic variables as described above.

Table 1. The environmental variables considered in this study. The five variables used for the hyperelements (HE) are marked with an asterisk and the transformations and categorisation of those variables are shown.

	Brief description	Transformation for HEs	Categorisation for HEs
Altitude			
Apan *	Potential evaporation	none	9 equal intervals
Continentality *	Measure of the extreme of temperature (T) (Jan max T minus July min T)	none	8 equal intervals except last interval is larger (contains very few records)
Coefficient of variation of annual precipitation (CVAP)	Inter-annual variation in rainfall		
Degree days (Winter)	Cumulative number of degree days between April and September, above a threshold of 10ºC.		
Degree days (Summer) *	Cumulative number of degree days between October and March, above a threshold of 10ºC.	Log(10)	7 categories based on natural breaks (Jenk's algorithm)
Frost days *	Number of days per year with heavy frost	none	Log(2)
Geology	Simplification of bedrock types: well-compacted sandstone, basalts, shales, granites, recent sands, limestone, silcretes and conglomerates		
Mean annual precipitation *		square root	8 equal intervals except large interval is larger (contain very few records)
Mean annual temperature			
Rain concentration	Measure of the spread of rainfall over 12 months		
Rain seasonality	Categorical description of when the most rain falls (5 categories) or all year rainfall.		
Soil fertility	Product of clay content and base status		
Solar radiation (January)	Considers solar radiation, and air temperature and temperature range of an area		
Solar radiation (July)	As above		
Temperature max (January)	Mean of the daily maximum temperatures in January		
Temperature min (July)	Mean of the daily minimum temperatures in July		

Mantel test

A Mantel test was used to test for a correlation between phylogenetic relatedness and habitat. A pairwise patristic distance matrix was calculated from the DNA matrix and phylogeny from Galley and Linder (2007) in PAUP (Swofford 2002). We scored the habitat parameters for each species using the upper and lower confidence intervals of the data recorded from each specimen. The variables are shown in Table 1 (except Geology). The values for each variable were range standardised and were used to calculate a pairwise distance matrix (average

taxonomic distance as implemented in NTSYSpc 2000-2003). The Mantel Test was calculated between these two matrices.

Bioclimatic units, the 'hyperelements'

The five selected environmental variables were transformed, categorised and combined to create bioclimatic units. The transformations and categorisations are shown in Table 1. Transformations were carried out to make the distribution of the variable as close to normal as possible. Variables were then categorised to maximise the discriminatory power the variable. For example, a log(2) categorisation of "frost days" was used because we expect that plants which have little or no frost will be more sensitive to the number of frost days than those which experience a lot. For each variable between 7 and 9 categories were made. The n categories of variable 1 were assigned values from 1,2…to n; of variable 2 from 10, 20…to 10*n; etc. for all the variables. The variables were combined by summing these five category values to obtain a five digit code. Each minute by minute cell in Southern Africa and each plant record were coded identically. The five digit code describes the bioclimatic attributes of a point in geographical space, or of a plant record, and is called a 'hyperelement' (HE). The hyperelements of a given species together represent the bioclimatic envelope of that species.

Regions and sub-regions

The diversity of the habitat available in the landscapes was calculated at the regional scale (the Cape and the Drakensberg) and a sub-regional scale (see below). The boundary used to characterise the Cape was taken from extent of the Broad Habitat Units (Cowling & Heijnis 2001). For the Drakensberg Region the extent of the following vegetation types (taken from the VEGMAP beta 4.0, National Botanical Institute, South Africa) was used: Southern Drakensberg Highland Grassland, Northern Drakensberg Highland Grassland, Lesotho Highland Basalt grassland, Drakensberg-Amatole Afromontane fynbos, Drakensberg Afroalpine heathland, Drakensberg montane shrubland, Drakensberg wetlands, Stormberg Plateau grassland, Ukhalhlamba basalt grassland, Western Lesotho basalt shrubland, Drakensberg wetlands, and Lesotho mires.

For a smaller landscape level four areas in the Cape and five areas in the Drakensberg were selected (see Figure 1). These are, in the Cape: the Cederberg, Hexrivierberg, Langeberg

and Cape Peninsula, and in the Drakensberg: Mount aux Sources, Cathedral Peak, Cathkin Peak, Sani Pass to Bushmans Nek and Naudesnek to Lundeans Nek. These sub-regions are well collected and the species lists each area is assumed to be complete.

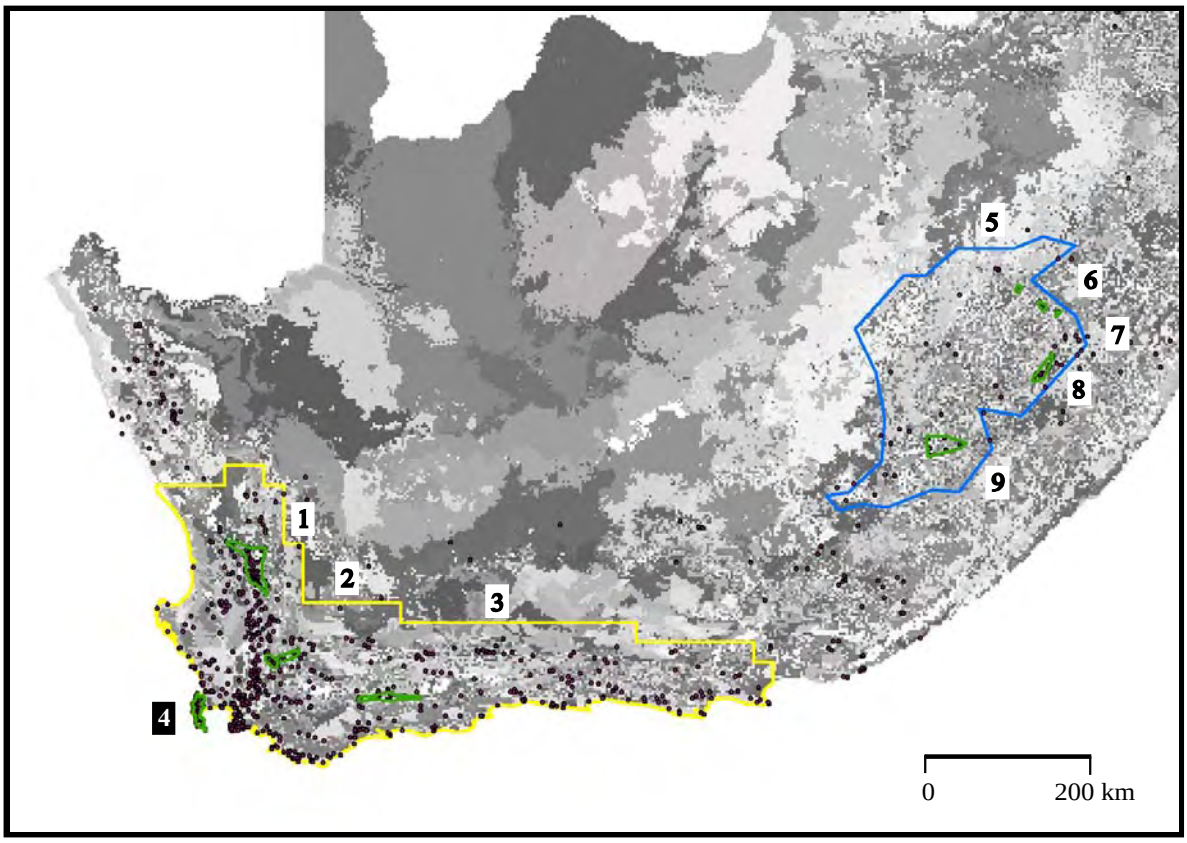


Figure 1. Map showing the unique hyperelements (shades of grey), the outline of the Cape (yellow) and Drakensberg region (blue) as used in the study and the *Pentaschistis* clade localities (pink spots) in South Africa. The four and five sub-regions (green polygons) are: 1, Cederberg; 2, Hexrivierberg; 3, Langeberg; 4, Cape Peninsula; 5, Mount aux Sources; 6,, Cathedral Peak; 7, Cathkin Peak; 8, Sani Pass to Bushmans Nek; 9, Naudesnek and Lundeans Nek.

We consider delta diversity as the turnover of species between the four and the five sub-regions. This follows closely the definition of Whittaker (1977), although an element of beta-diversity may occur due to habitat differences between the sub-regions (Linder 1985; Cody 1986). We consider the species richness in each of these sub-regions to represent gamma diversity, which also follows the definition of Whittaker (1977), and is a function of both alpha and beta diversity (Whittaker *et al.* 2001). The scale of the study precludes the study of alpha and beta diversity specifically.

Characterising the species

The number of unique HEs each species occupies was used to estimate the niche breadth of each species. To estimate the geographical occupancy of each species, a polygon was drawn around the locality points and the area calculated from this. For both measures, the species from the Cape and the Drakensberg, respectively, were pooled and compared. Species which occupy less than 600 km^2 are termed extremely range restricted species. Those which occupy more than this but do not occur in all four or five of the sub-regions, we term partially range restricted.

A bias in sampling was tested for by comparing the mean number of records (ln transformed) per species for both regions, as well as a Mann-Whitney U test on the untransformed data.

Gamma diversity

Species

Species lists were compiled for the Cape and Drakensberg Range as well as the four and five well-collected sub-regions. The lg species richness of the sub-regions was plotted against lg area and a regression line plotted through the nine points. The standardised residuals of the regression were compared to test whether the sub-regions of either region have more or less species expected per area.

To compare the co-occurrence of species between within each region we measured shared occupancy of habitats. The number of species occupying each unique HE in the Cape, and in the Drakensberg was counted. This however considers the predicted distribution, not the actual distribution; HEs may be repeated across the landscape, so that even if two species occupy the same HE type, they do not necessarily co-occur. Co-occupancy without co-occurrence would be due to geographical replacement. We tested whether or not this is an important factor in the Cape by repeating the analysis but restricting the records and HEs considered, to each of the four sub-regions. The values were then pooled and compared with those for the entire Cape region.

Habitats

The amount of habitat available in each of the two regions was first compared using the individual environmental variables that are shown in Table 1. For each variable, the standard deviation between the two regions was compared.

The diversity of habitat space in the two regions was also estimated using the HEs as units. The number of unique HEs in both regions was counted to estimate the amount of available habitat. A regression was used (lg HEs = lg species) was used to investigate the proportion of species richness in each sub-region which is explained by the number of HEs.

To estimate the amount of this habitat that is available to *Pentaschistis*, the number of unique HEs that are occupied by *Pentaschistis* only, in each region was counted. To evaluate the importance of this factor we predicted how many species we would expect in the Drakensberg if the occupancy rate (of habitats) were the same as in the Cape. We used the following equation: [area (=number of HEs) * proportion of HEs occupied] * factor 'Y' = number of species. We used the Cape area, proportion and species number to solve for Y. The equation was then applied to the Drakensberg area, substituting the 'proportion occupied' for the Cape proportion occupied, to obtain a predicted number of species.

Delta diversity

Species

Turnover in species within the Cape and within the Drakensberg was estimated by pairwise comparisons of the four and the five sub-regions, respectively, using the B-value (Wilson & Shmida 1984). Between two areas, the B-value is the total number of taxa unique to each area divided by the pooled species richness of the two areas. The mean B-values between the Cape areas and the Drakensberg were then pooled and compared. To test the influence of range restricted species on the delta diversity, taxa which are present in one area only were removed, and the B-values re-calculated.

A second estimate of species turnover is the cumulative number of new species encountered as the four or the five sub-areas are visited in succession. This was then be adjusted for area and the accumulation rates between the two regions compared, by testing for an interaction term between cumulative area and region (Cape or Drak). ANOVA: cumulative species = cumulative area + Cape/Drak + cumulative area*Cape/Drak.

Habitats

The heterogeneity of both areas at a local to medium scale (within an area of ~12 km^2) was investigated using a 'roughness' measure. This was calculated for each variable using neighbourhood statistics in GIS. Each minute by minute cell (the focal cell) is considered, along with its eight neighbouring cells. The focal cell receives a value that is the range of the values in the neighbourhood. If the nine cells are very variable the focal cell receives is high score whereas if the nine cells are identical the focal cell receives a '0' score. The scores of the focal cells were pooled across the Cape and across the Drakensberg, and the mean of the two distributions compared.

Habitat heterogeneity was also calculated for the combined environmental variables, using the HEs as units. The cumulative number of HEs encountered as one visits the four or five sub-areas in succession was recorded. This was corrected for area and compared to the cumulative number of unique species (see above) to test if the accumulation in HEs accounts for the accumulation in species. The rates of HE accumulation between the two regions were compared by testing for an interaction effect, as described above. ANOVA: cumulative species = cumulative HEs + Cape/Drak + cumulative HEs*Cape/Drak. This compares heterogeneity between distinct landscapes.

Species turnover may be due to habitat turnover or to other disequilibrium effects meaning a species does not occupy its estimated potential range. To discriminate between these two factors, we recorded the discrepancy between the actual and potential distribution of every species. The potential distribution was calculated using the HEs from all of the records for a given species. We searched for these HEs within the Cape or within the Drakensberg. The four or five sub-regions were then scored for presence or absence of predicted distribution, and this was compared to the (recorded) presence or absence. The pooled prediction errors of the Drakensberg and the Cape species were then compared. We also tested the extent to which range restricted species were restricted because of a restricted distribution of their niche, within the Cape. The Cape dataset was split into species occupying a range less than or greater than 600km^2, and the predictability of these two groups was compared. If range restricted niches cause species to be range restricted, these species should be better predicted than more widespread species.

Statistical tests

Data comparing aspects of the Cape and the Drakensberg were compared using a t-test of the mean if distributions were approximately normal (usually following a log(10) or natural log transformation). Alternatively, a Mann-Whitney-U test was used. Distributions were also analysed using skewness and kurtosis measurements. All statistical tests were carried out in SPSS (2003). The similarity matrices were calculated in NTSYSpc (2000-2003).

RESULTS

Phylogenetic and ecological correlation

There is no correlation between patristic distance and the habitat preference of each species (Mantel test; r2 = -0.0163, P = 0.4047, lower confidence interval of the habitat variable; r2 = -0.00812, P = 0.4517, upper confidence interval of the habitat variable).

Species richness (regional level)

The Cape contains 65 species, the Drakensberg Range, 11. Using the areas defined in Fig. 1, the Cape has 0.000530 species per km^2 and the Drakensberg has 0.000149 species per km^2.

Characterising the species

Species niche breadth

The number of log(10) HEs for each species in the two regions is shown below (Fig. 2). The means of the distributions are similar (t-test: df = 1, P = 0.871), as are the distributions of the HE number; both regions have a similar proportion of species which occupy less than 10 HEs (0.43 for the Cape, 0.42 for the Drakensberg), and less than 20 HEs (0.71 for the Cape, 0.75 for the Drakensberg). The species in the Cape have a similar estimated niche breadth as those in the Drakensberg but an important difference is the 13 Cape species which occupy one or two hyperelements.

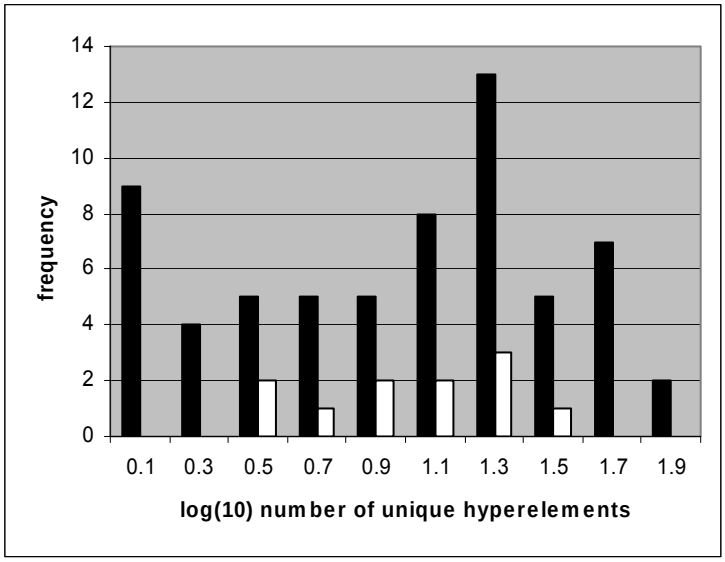


Figure 2. Histogram of the log(10) unique HEs occupied by each species in the Cape (black bars) and the Drakensberg (white bars). Mean value of the Cape distribution = 0.952, standard deviation = 0.573; mean value of the Drakensberg distribution = 0.982, standard distribution = 0.346.

Species range size

The mean log(10) area occupied by each species in the two regions differs, with Cape species occupying on average smaller ranges than the Drakensberg species (t-test: df = 1, P = 0.060). 68% of the species occupy less than 1 square degree, compared with 45% in the Drakensberg. Comparing the distributions of the species ranges in each region is instructive. The distribution of the Cape species range sizes is more skewed than that of the Cape, illustrated by the skewness and kurtosis values (see Table 2 and Figure 3). The mean skewness of the Cape distribution is greater than twice the standard error (an indication of significant departure from symmetry, SPSS) and the kurtosis is positive indicating a distribution more spread out than a normal distribution. The Drakensberg distribution is not significantly skewed. Many of the Cape species have been collected from single or geographically clustered localities. Although this might reflect true distribution (McDonald & Cowling 1995) it might also be in some cases artefact from under-sampling. We therefore re-calculated the skewness statistics with these species removed (Table 2) and the results are similar. More Cape species occupy smaller range than Drakensberg species.

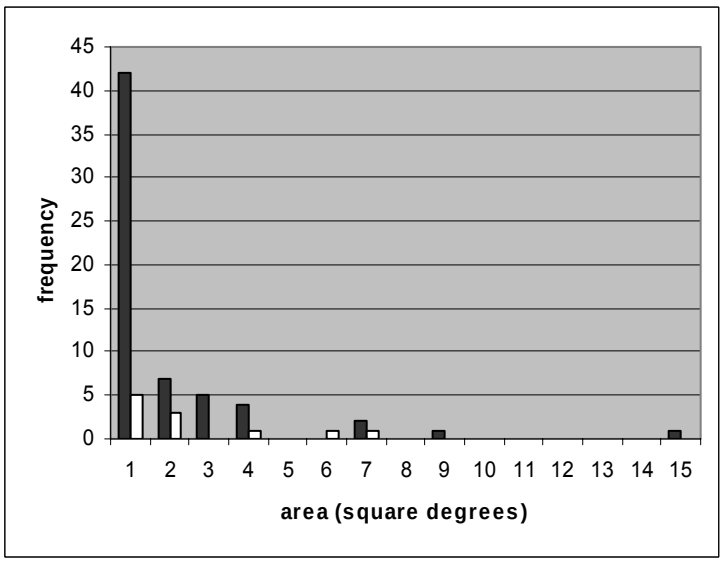


Figure3. Histogram of area of each species for the Cape (black bars) and the Drakensberg (white bars). 68% of the species occupy less than 1 square degree.

Table 2. Skewness and kurtosis of the area for each species in the Cape and the Drakensberg.

	N	min	max	mean	SD	skewness	S.E.	kurtosis	S.E.
Area (square degrees) of Cape species	63	.0060	14.69	1.280	2.44	**3.60**	.302	15.628	.595
Area (square degrees) of Cape species with the single-locality records removed	51	.0071	14.69	1.58	2.63	**3.23**	.333	12.827	.656
Area (square degrees) of Drak. species	12	.0448	6.20	2.290	2.29	.74	.637	-1.046	1.232

Species sampling

The natural log (ln) transformation of the number of records for each is shown in Figure 4. The mean ln number of records is similar between the two regions (t-test, P = 0.104). A Mann-Whitney U test of the raw data shows the medians of the two distributions are also similar (P = 0.294). Again, in the Cape there are species which were recorded only once. To check how much this potential bias might have influenced the results, we removed these six species and repeated the tests. The results of both tests (t-test, P = 0.146; Mann-Whitney U test, P = 0.547) suggest no significant bias. We observe no collecting bias between the two regions.

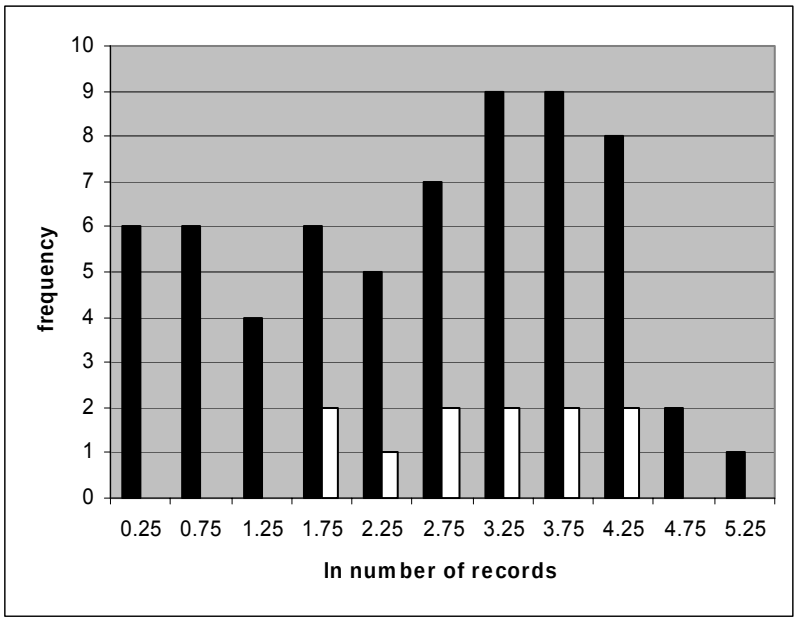


Figure 4. Ln number of records per species for the Cape (black bars) and the Drakensberg (white bars). Mean value for the Cape distribution = 2.567, standard distribution = 1.434; mean value for the Drakensberg distribution = 3.106, standard deviation = 0.868.

Landscape diversity

Species

There are 45 species in the four sub-regions of the Cape and eight species in the five sub-regions of the Drakensberg. A linear regression of lg species against lg area has an R^2 of 0.467. The standardised residuals of the Cape are greater than those of the Drakensberg (Mann-Whitney U test, P = 0.013); the sub-regions in the Cape have more species than those in the Drakensberg (see Appendix 2), adjusting for area (see Fig. 5)

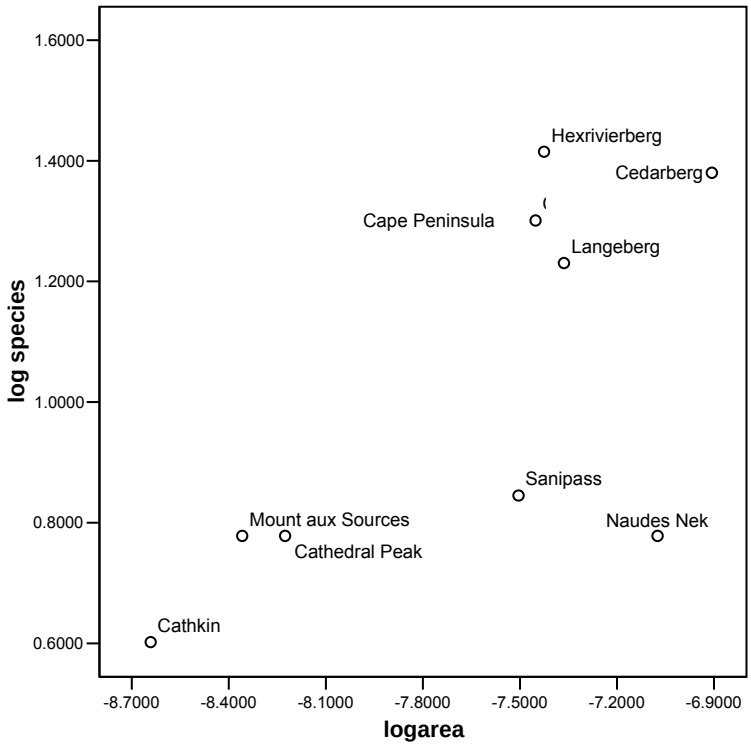


Figure 5. The log (10) species richness of each of the sub-regions (upper four are from the Cape, lower five are from the Drakensberg), plotted against log (10) area.

The packing of species due to species ecological overlap was estimated by measuring the numbers of species which occupy each unique HE (Figure 6a, b). The distributions are very similar (see the skewness and kurtosis measures in Table 3), but the histograms show that the scales of these distributions are very different. In the Drakensberg the maximum number of species potentially occupying a HE is six, whereas in the Cape 12% of the unique HEs are occupied by more than six species. Although we would expect that the Cape has potentially more species per HE (simply because it has more species), the Drakensberg contains eleven species of *Pentaschistis*, yet the maximum value of co-occupation is much lower.

To distinguish between geographical replacement and actual co-occupancy in the Cape the calculations were repeated but with a sub-set of the data, within which we would not expect any geographical replacement. The numbers of species occupying each HE are shown in Figure 6c. A Mann-Whitney U test shows that the difference between the occupancy within the region or the sub-regions is not significant (P = 0.327) suggesting that geographical

replacement does not play a role. More species in the Cape are able to co-occur in a given HE than in the Drakensberg.

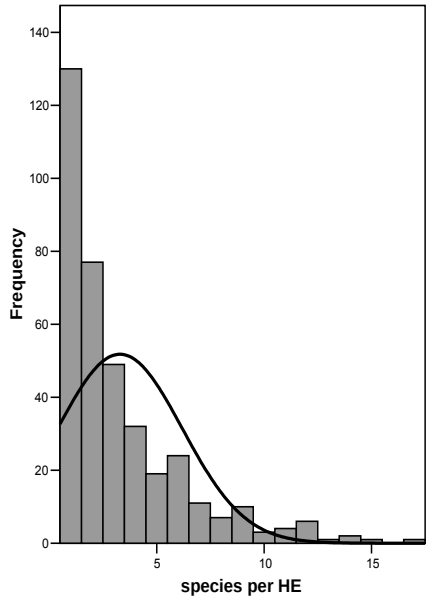


Figure 6a. Histogram of the number of species which occupy each unique HE in the Cape.

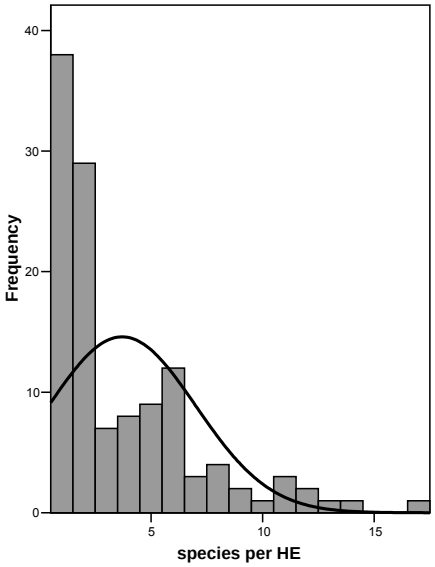


Figure 6c. Histogram of the number of species which occupy each unique HE in the Cape, but species and records are considered restricted to the four sub-regions (the data for the four sub-regions are pooled).

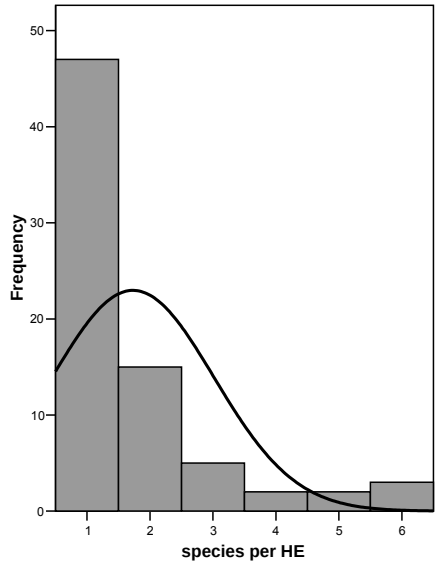


Figure 6b. Histogram of the number of species which occupy each unique HE in the Drakensberg.

Table 3. Skewness and kurtosis of the number of species per unique HEs in the Cape and Drakensberg.

	N	min	max	mean	S.D.	skewness	S.E.	kurtosis	S.E.
Species per HE (Cape)	406	1	17	3.26	2.907	**1.772**	**.125**	**3.264**	**.250**
Species per HE (Drak.)	89	1	6	1.73	1.277	**2.120**	**.277**	**4.047**	**.548**

Habitats

The environmental variation of the Cape and the Drakensberg is similar. The mean and the range of each environmental variable are shown in Appendix 4. The Cape has a greater variable range in seven cases, and the Drakensberg in eight. This suggests neither of the regions have more habitat available than the other.

The results considering the variables combined (as HEs) are shown in Table 4. The Cape and the Drakensberg again have a similar habitat range as the number of unique HEs per area is similar for both regions. However, the number of unique HEs which are occupied by *Pentaschistis* is much greater in the Cape than the Drakensberg, as would be predicted by area. The contrast between the number of unique HEs and those occupied by *Pentaschistis* is significantly different (Fisher exact test, $P < 0.001$). This is reflected by the greater proportion of *Pentaschistis*-occupied hyperelements per area, in the Cape than in the Drakensberg. If the Drakensberg had the same proportion of the total habitat occupied by *Pentaschistis* as the Cape, we would predict 34 species in the Drakensberg, over three times the current number.

Within the sub-regions, lg HE number explains 0.631 of the variance in lg species number, but in an exponential model, 0.671. It must be noted that the Cape sub-regions tend to have more HEs per sub-region than those in the Drakensberg, and that the exponential effect of lg HEs on species number might therefore be an effect of this.

Table 4. The area (number of minute x minute points), number of unique hyperelements (HEs), number of unique HEs occupied by *Pentaschistis*, and the number of species, in the Cape and the Drakensberg. Figures in the bottom half of the table are derived from these.

	Cape	Drakensberg
Total number of HEs (~area)	42143	22264
Number of unique HEs	1847	832
Number of unique HEs occupied by *Pentaschistis*	382	83
Number of taxa	65	11
Unique HEs per total number of HEs (area)	0.044	0.037
Unique HEs per species	29.32	75.64
Unique *Pentaschistis* occupied HEs per species	5.88	7.54
Unique *Pentaschistis* HEs per area	0.0091	0.0037

Delta diversity

Species

The B-values in the Cape are higher than those in the Drakensberg reflecting a higher species turnover between sub-regions in the Cape (Fig. 7, Mann-Whitney U test, $P < 0.001$). This is reflected by the distribution of the species in the species lists of the sub-regions (see

Appendix 1); only one of the five areas in the Drakensberg has a species not found in the other sub-regions, whereas the sub-regions in the Cape have between three and six unique species each. Furthermore three out of seven of the Drakensberg species are found in all sub-regions, compared to only five out of 44 of the Cape species.

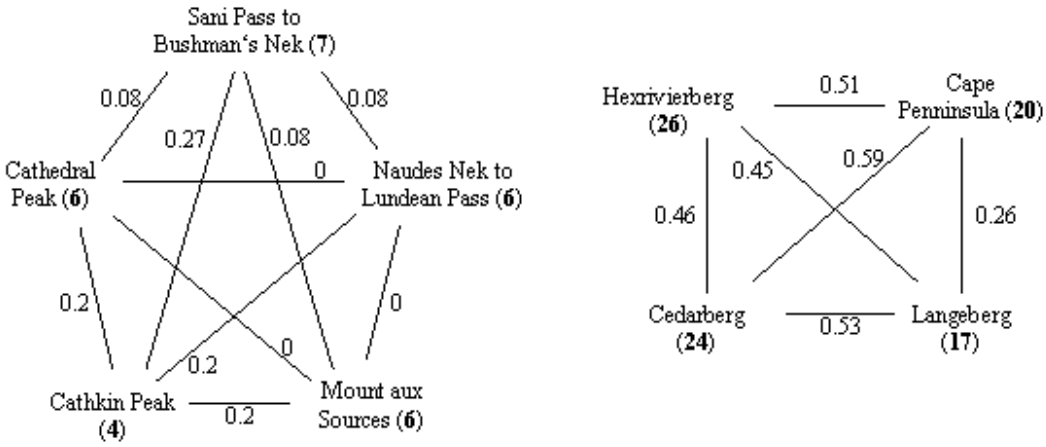


Figure 7. Sub-regions of the Drakensberg (left) and the Cape (right). Numbers in brackets are the number of species recorded for each sub-region. Numbers along the lines are the B-values between the sub-regions.

The B-values for the reduced dataset, where taxa present in only one of the sub-regions were removed, are also higher in the Cape than the Drakensberg (Mann-Whitney U test, P = 0.004). This suggests that very range restricted species do not fully explain the higher delta diversity in the Cape.

The accumulation of species from one sub-region to the next occurs at a faster rate in the Cape than in the Drakensberg, adjusting for area (interaction term of the univariate ANOVA, P = 0.003).

Habitats

Habitat heterogeneity, calculated using the individual variables, shows that at a local scale the two regions are similarly heterogeneous. The Cape is more heterogeneous than the Drakensberg for seven variables, but less than it for eight variables (see Appendix 3).

The habitat turnover was also tested using the combined variables (HEs), restricted to the sub-regions. The interaction term of the ANOVA is non-significant (P = 0.244), showing that from one sub-region to another, the turnover of HEs (adjusting for area) is not significantly different between the two regions.

The distribution disequilibrium of species within each region was evaluated by comparing the predicted occurrence of each species with its recorded occurrence, in each of

the four or five sub-regions. The modelling was much more accurate in the Drakensberg than in the Cape (Mann-Whitney U test of the prediction failures, P = 0.032). There was only one wrongly predicted species in the Drakensberg, compared to several species in the Cape which were wrongly predicted, and for several sub-regions (see Fig. 8). Figure 9 a , b, c, d are examples of the predicted distributions of nine species in the Cape or the Drakensberg. The predictability of species with a restricted range was not significantly different than non-range restricted species in the Cape (Mann-Whitney U test, P = 0.397).

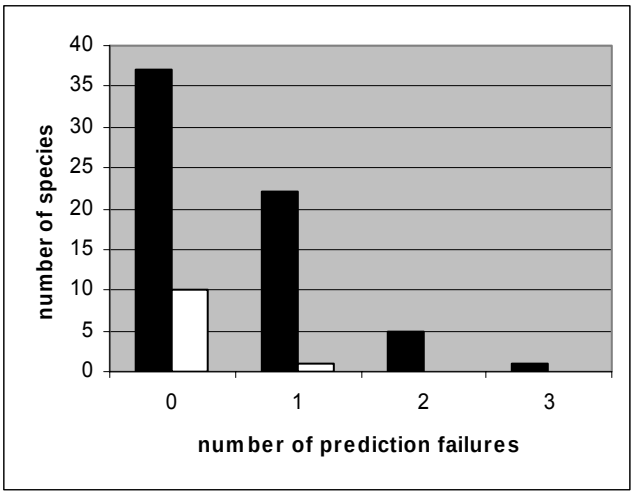


Figure 8. Histogram of the prediction failures from the predictive modelling for species from the Cape (black bars) and the Drakensberg (white bars).

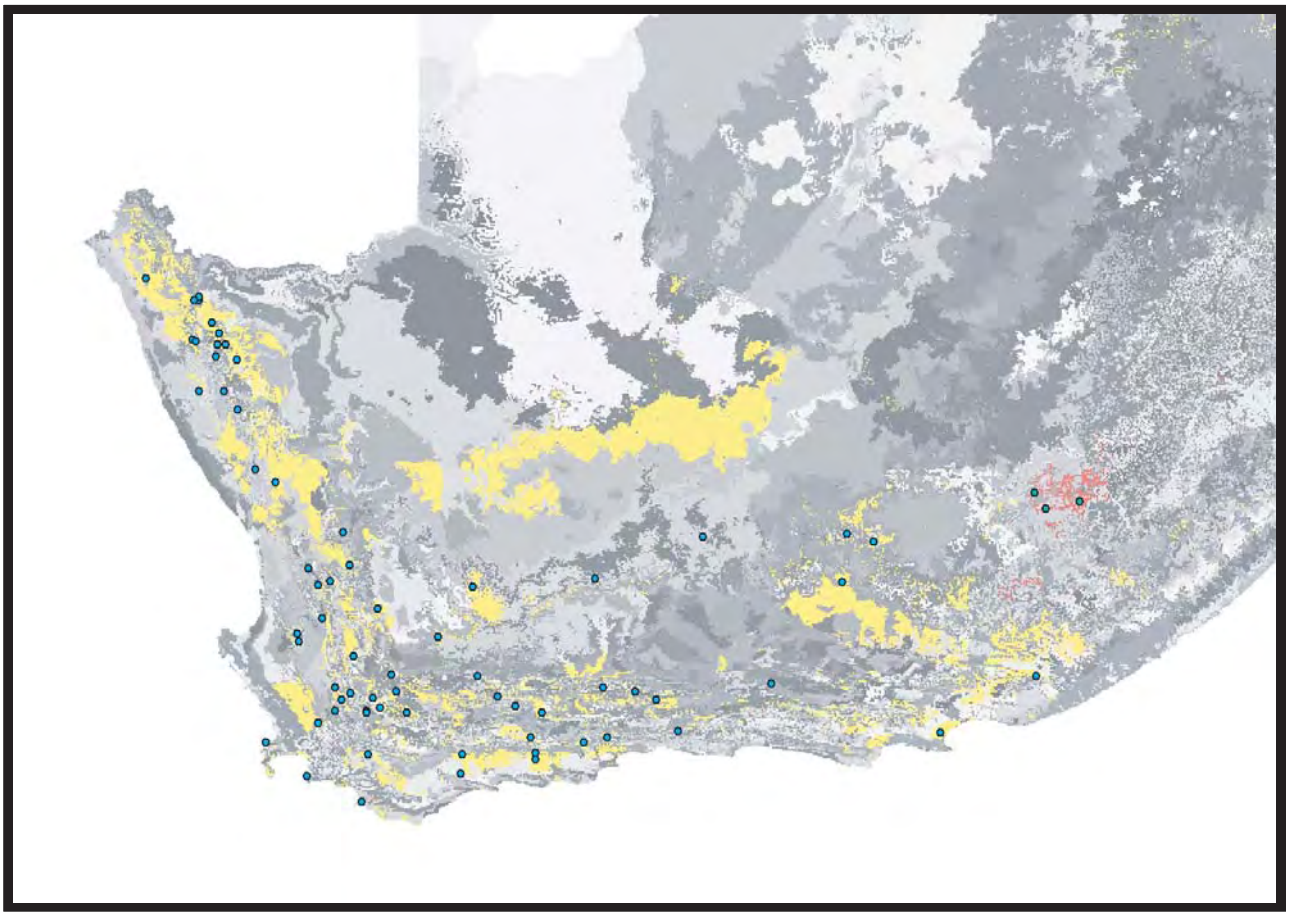


Figure 9a. Predicted (yellow) and actual (blue spots) distribution of *Pentaschistis airoides* ssp. *airoides* and predicted (red) and actual (turquoise spots) distribution of *Pentaschistis microphylla.*

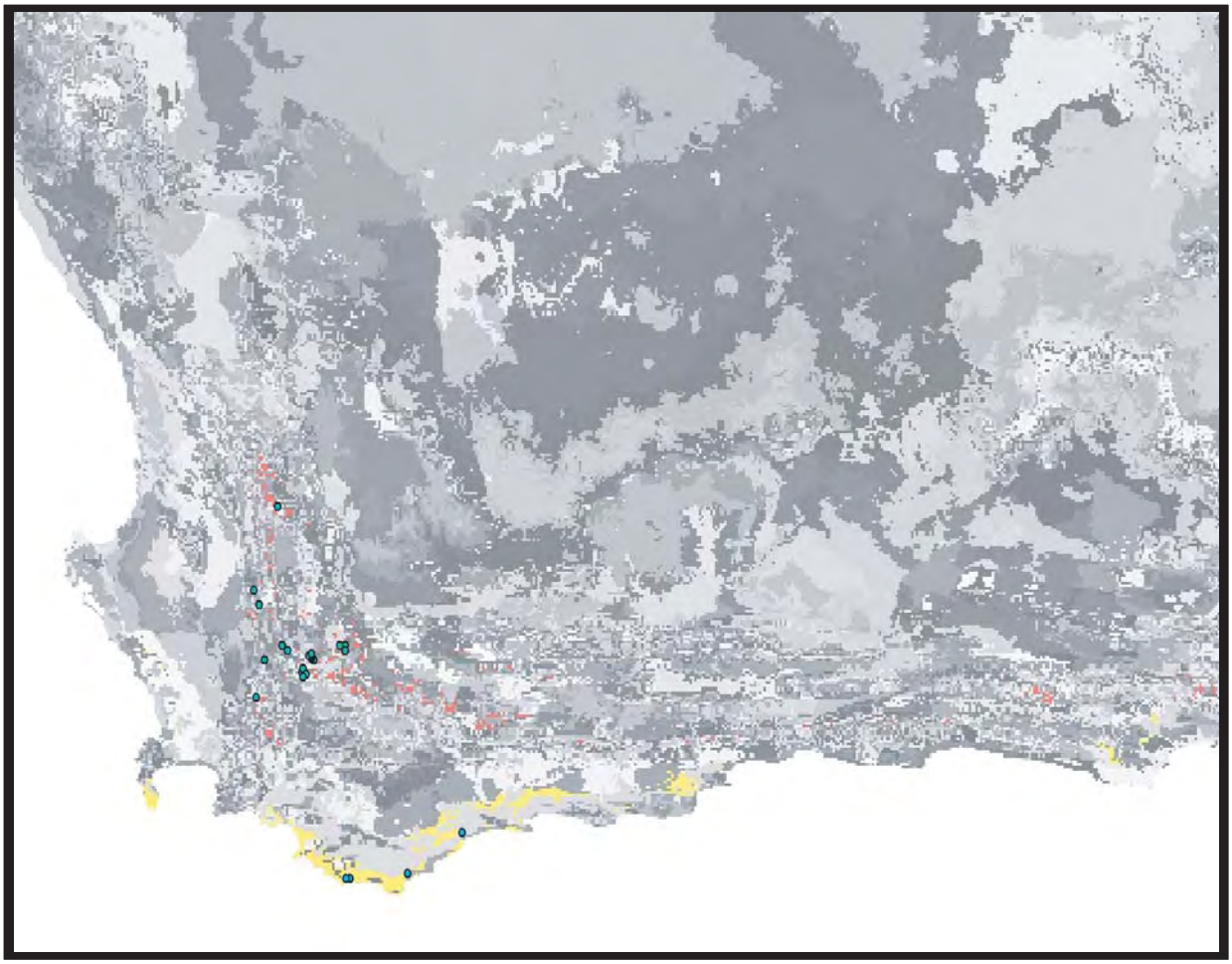


Figure 9b. Predicted (yellow) and actual (blue spots) distribution of *Pentaschistis scandens* and predicted (red) and actual (turquoise spots) distribution of *Pentaschistis alticola.*

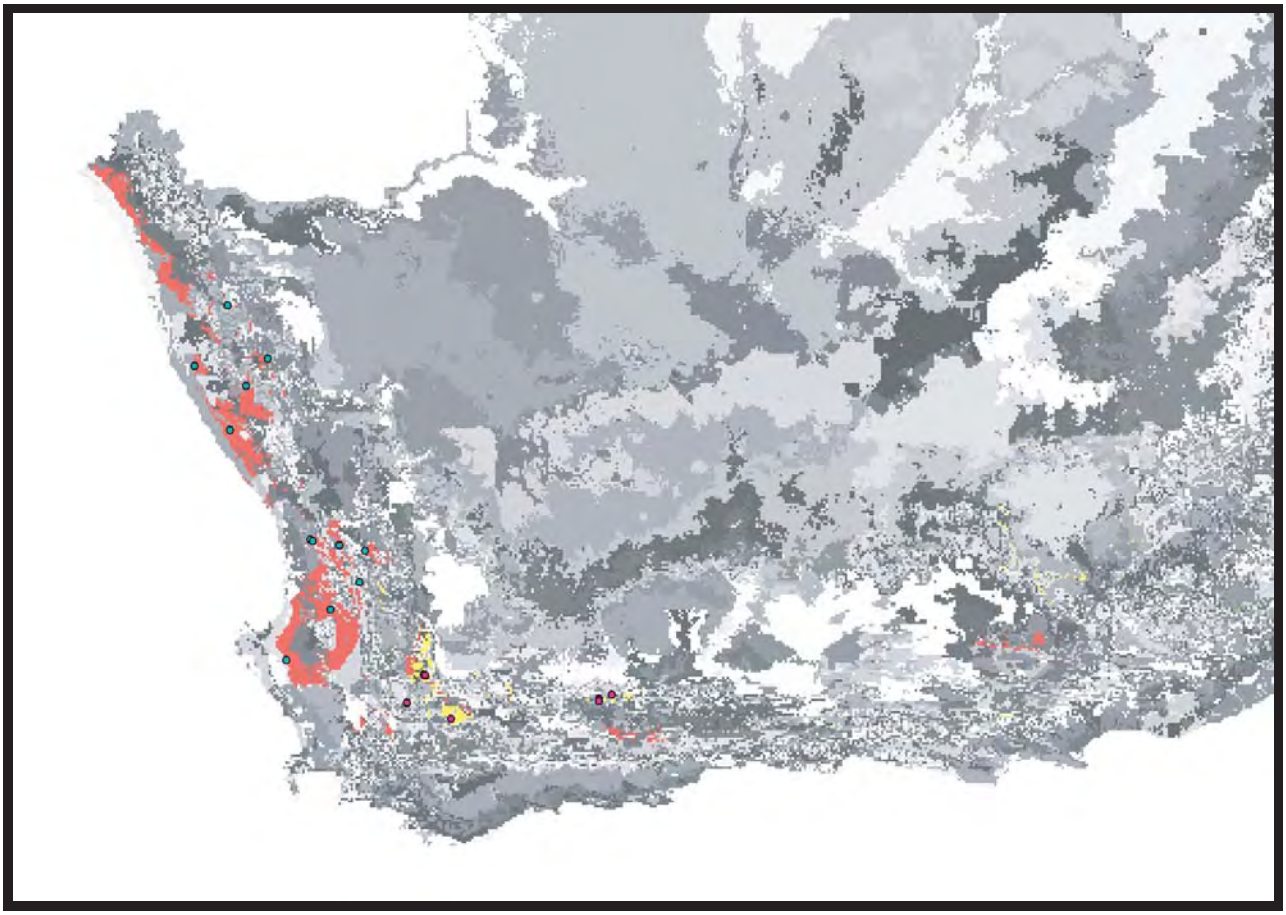


Figure 9c. Predicted (yellow) and actual (pink spots) distribution of *Pentaschistis horrida* and predicted (red) and actual (turquoise spots) distribution of *Pentaschistis capillaris.*

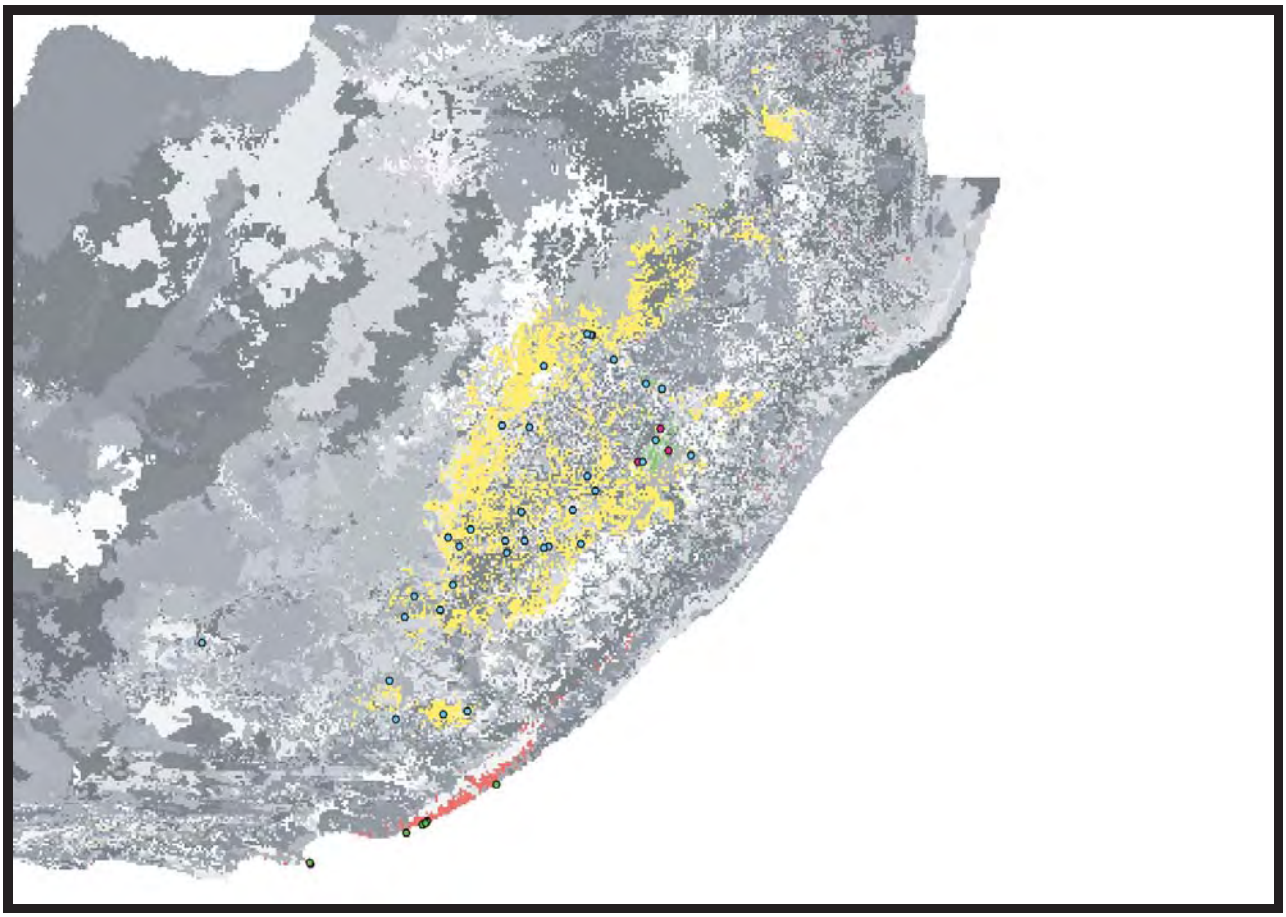


Figure 9d. Predicted (yellow) and actual (blue spots) distribution of *Pentaschistis setifolia* and predicted (red) and actual (green spots) distribution of *Pentaschistis horrida*, and predicted (green) and actual (pink spots) distribution of *Pentaschistis praecox.*

DISCUSSION

We used *Pentaschistis* to investigate the contrasting species richness between the Cape and the Drakensberg. Ricklefs and Schulter (1993) emphasise that geographical, historical and evolutionary factors need to be considered in addition to ecological factors, to understand species richness. The *Pentaschistis* clade has eleven species centred in the Drakensberg but only two of these are closely related (see Fig. 10) and the species there are neither especially young compared to the Cape species (Galley & Linder 2006a). This excludes any species-age or lineage-specific differences between the two regions, which leaves only factors relating to the regions themselves, as the difference between the taxa of the Cape and the Drakensberg. We concentrate on the role of habitat heterogeneity, suitability and partitioning, and distribution ranges in accounting for the species richness of the Cape.

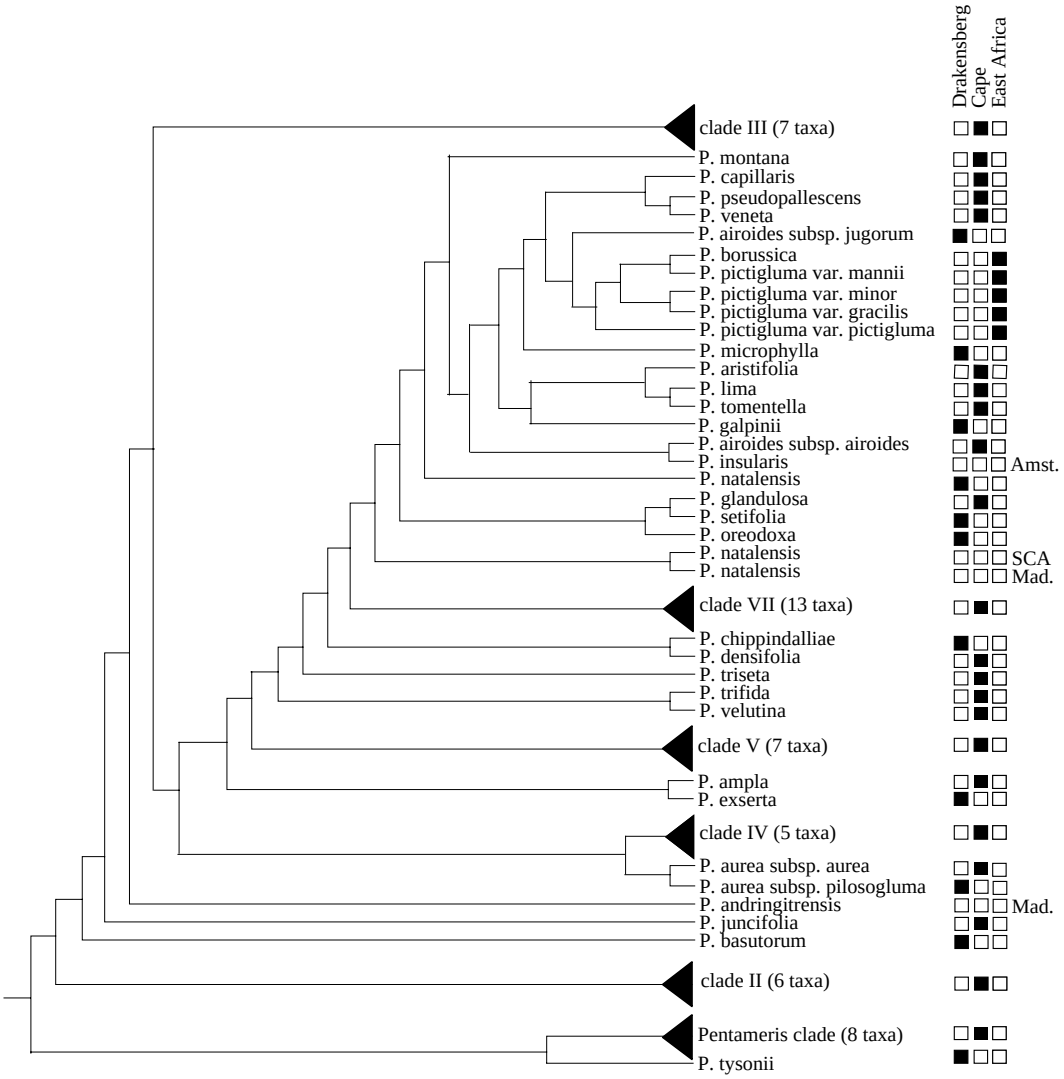


Figure 10. Phylogeny of the *Pentaschistis* clade showing the distribution of each species. Additional areas: Amst., Amsterdam Island (South Indian Ocean); Mad. Madagascar; SCA, South-Central Africa. Adapted from (Galley & Linder 2006a)

Some methodological issues

Regions that are mega species rich are difficult but important to explain. GIS can be used to characterise the habitat occupied by a species and there has been a recent increase in the use of this to explain co-existence and species diversity (Guisan & Thuiller 2005). The number of occurrence points per species affects the precision and accuracy of the habitat characterisation, and predictive modelling (Stockwell & Peterson 2002). We present a dataset with a mix of well collected and poorly collected species; in the Cape dataset there are 27 species with fewer than ten records, in the Drakensberg, two. Discarding species with few records to reduce error associated with small samples as others have done (e.g. Broennimann *et al.* 2006), would miss out what here are potentially the most important species. We minimised the under-sampling effect by two methods. Firstly we decreased the precision by using broad categories of variables to construct our comparative habitat units, the hyperelements. It has been demonstrated that predictive modelling based on coarse habitat categories is more successful than that based on narrow habitat categories when dealing with small datasets, including those with between ten and 20 data points especially (Stockwell & Peterson 2002). Secondly we concentrated many statistics, including evaluating disequilibrium and calculating delta diversity, on nine well-collected areas in the Cape and Drakensberg, which should minimise false absences.

Many regions that are very species rich will have such mixed datasets as this, where some species are well represented, and others not. We argue that such datasets should not be discarded, yet given the time and cost of collecting biological data, sampling is not likely to increase dramatically in the next few years. Being able to use these current datasets is therefore important.

The relative poor prediction power of the bedrock dataset deserves special mention since edaphic factors implicated in explanations of high species turnover in the Cape. In *Pentaschistis* many species are restricted to one or two bedrock types each. However, these tend to be the same two bedrock types (sandstone and granites in the CFR, basalt and shale in the Drakensberg) and these are very widespread in the two regions. This does not preclude bedrock type as an important determinant of species occurrence at a local area, but the effect is that bedrock type is a poor prediction variable at the region-level.

Explaining the gamma diversity

We measure species richness at a smaller landscape level (sub-regions from 25 to 1295 km^2), showing that it is much higher in the Cape than the Drakensberg. It is generally expected that for similar areas there is a linear relationship between the lg number of species and area or lg area (Rosenzweig 1995). We demonstrate that this is not the case for *Pentaschistis*, as sub-regions have more species in the Cape than expected from area alone. The Cape is recognised as having long and steep habitat gradients of soil type (Goldblatt 1997; Goldblatt & Manning 2002), precipitation (Linder 1991; Simmons & Cowling 1996), phytochorological assembly (Cowling 1983), fire regime (Cowling 1987), and these are possibly related to the high species richness (Simmons & Cowling 1996). We measured the habitat diversity of the Cape and the Drakensberg using individual environmental variables and found that the two regions have a similar amount of habitat space.

With few exceptions (e.g., Cowling & Lombard 2002) investigations have treated environmental variables individually, but this is not the perspective of the plants. We use hyperelements as estimates of habitats to circumvent this. However the results are similar; accounting for area, there is roughly the same number of unique hyperelements available in both regions. Furthermore we tested whether the number of hyperelements predicts species richness in the sub-regions. The relationship between the two variables is significant but it is an exponential relationship. This means that there is something in addition to habitat diversity which causes a difference in species richness between the Cape and the Drakensberg. Cowling and Lombard (2002) used community diversity as a surrogate for habitat diversity and conclude that this shows no appreciable variation in relation to species diversity of sites. The question remains, what else explains higher landscape and so regional species richness in the Cape?

Do Cape species have smaller niches?

A smaller niche breath would mean that more species can pack into a given unit of habitat (see Marquet *et al.* 2003, and references therein). There is no evidence that species in the Cape have a smaller niche breadth than those in the Drakensberg, as estimated by the hyperelements. It is however possible that the habitat in the Cape is more heterogeneous than our hyperelements can measure. Such division into smaller niches would support more species per habitat unit, which in turn would allow more species to fit in a landscape. We test and discuss this in the following sections.

Do Cape species overlap more in habitat space?

We tested for the ecological overlap of species by counting how many species can fit into one hyperelement. Hyperelements in the Cape were occupied by more species than in the Drakensberg. Species may appear to share hyperelements for two reasons; they share the hyperelement type, but do not co-occur (they are in geographically distant areas), or they are sympatric. The first possibility is a result of geographical replacement, whereby competition could restrict them from occupying their full range, but we demonstrated that this is not responsible for the higher ecological overlap of *Pentaschistis* in the Cape. This leaves the second option, that in the Cape more species are able to co-occur in a given habitat unit.

Hyperelement heterogeneity in the Cape and the Drakensberg

The higher number of coexisting species in the Cape hyperelements suggests that each hyperelement is more heterogeneous there than in the Drakensberg. It is possible that those in the Cape vary more in macrohabitat parameters which we did not include, or that they include more microhabitats (Linder 2005). Many *Pentaschistis* species are associated with microhabitats such as drainage (*P. pallescens*), exposed rock (*P. calcicola*) and soil or bedrock type (*P. longipes*, *P. calcicola*, *P. elegans*). Such characteristics are not possible to model using macrohabitat datasets such as the one used here and would need to be handled case by case. Niche size and niche parameters are in any case notoriously difficult to quantify (Leibold 1995; Marquet *et al.* 2003) but doing so will be important, as our data suggest that microhabitat differences allow more species to coexist within a landscape in the Cape.

There is an additional axis which might be important in packing species into areas in the Cape – the temporal niche due to fire. It is known that fire plays an important role in the Cape vegetation, and in allowing certain species such to persist across the landscape (Cowling 1987; Simmons & Cowling 1996). There are many species of *Pentaschistis* which are frequent one to several years after fire (*P. pseudopallescens*, *P. rosea* and *P. triseta*), but are less frequent (absent?) in more mature vegetation. Fire, as a spatially and temporally complex variable will be a challenge to disentangle, and further requires a contrast with the Drakensberg, whose vegetation is also affected by fire (Hilliard & Burtt 1987).

Do the Cape species use more of the available habitat?

Measures of habitat availability often assume that plants can occupy all habitats present, or all intermediate levels between the extremes of variables that are measured. Perhaps more

relevant, however, is the amount of habitat that is suitable, which is estimated by the proportion of unique HEs actually occupied by the clade. We calculated this and found a significantly greater proportion of available habitat which is occupied by *Pentaschistis* in the Cape, compared to in the Drakensberg. This is reflected by the similar number of *Pentaschistis* occupied hyperelements per species in both the regions (see Table 4) suggesting that occupied habitat explains much of the difference between the two regions. The difference in habitat usage has a second component however. There are several niches which exist in both the Cape and the Drakensberg, but which are only occupied (by *Pentaschistis*) in the former, for example stream banks (*P. capensis*) and rock ledges (*P. rigidissima*, *P. densifolia*). Not only is more of the habitat in the Cape suitable for *Pentaschistis*, but from this habitat, more of it tends to be occupied than in the Drakensberg.

Explaining the delta diversity

Species turnover between landscapes within the Cape is much higher than in the Drakensberg. Although there are some widespread species in the Cape (e.g. *P. colorata*, *P. curvifolia*, *P. glandulosa*), these are a minority, most are partially range restricted. In contrast, in the Drakensberg half of the species are widespread throughout the main range of *Pentaschistis*. This is demonstrated by the species lists of the sub-regions (see Appendix 2). Furthermore there are more severely range restricted species in the Cape; additional to those listed for the sub-regions, 16 species in the Cape which are severely range restricted, but only one extra in the Drakensberg. The high number of range restricted species, culminating in high local endemism is associated with the high turnover and species richness in the Cape (Kruger & Taylor 1979; Simmons & Cowling 1996; Cowling & Lombard 2002; Latimer et al. 2005). The question of delta diversity then becomes one of explaining these partially and severely range restricted species. We tested for habitat turnover at a local to medium scale – cell neighbourhood heterogeneity – and a sub-regional scale – habitat accumulation between sub-regions. There is no evidence that habitat turnover is higher in the Cape than in the Drakensberg. McDonald and Cowling (1995) and Cowling and Holmes (1992) typified range restricted species in the Cape as dwarf to low non-resprouting shrubs with short distance seed dispersal, possibly ant dispersed, but *Pentaschistis* does not fall into these categories. We address some other possibilities, in turn.

Firstly, a species may be range restricted because the niche which it occupies is also restricted. We estimated this by comparing the predictability of range restricted species with that of more widespread species. There is no evidence that niches are less widespread in the former than in the latter group. However there is a bias which might have caused this effect; widespread species are easier to correctly model if the model is evaluated by false presences (e.g. a species occurring in all four sub-regions of the Cape cannot be wrongly predicted). The bias would be against correctly predicting the range restricted species. Furthermore, it is also possible that range restricted species are endemic to range restricted microhabitats that are not included in our hyperelements. For example, range restricted bedrock types, which was not included in our model, but to which some species are endemic (e.g. *P. calcicola*, *P. triseta*), or an ephemeral (temporal) niche would be missed by such a macro-ecological study.

Secondly, a species might be range restricted because for contemporary or historical reasons it does not occupy its full distribution as is predicted by the habitats of recorded populations (e.g., due to slow rate of population expansion, Bennet 1988; migrational lag, Huntley 2006; or human and or fire disturbance, Tinner & Lotter 2006). We tested for this distribution disequilibrium by comparing the prediction success of the model for Cape and Drakensberg species. Species in the Drakensberg tend to fill their predicted ranges more than in the Cape, suggesting that disequilibrium is indeed higher in the latter region. It might be explained by geographical replacement of species in the Cape, but we demonstrate that this is probably not important for *Pentaschistis* in the Cape. Alternatively, Latimer et al.(2005) hypothesised that limited migration related to a "topographically fragmented" metacommunity combined with higher speciation rates, explains the high local endemism in the Cape. However we do not think this model adequately explains the range restricted *Pentaschistis*. The plants are presumably wind pollinated and dispersed. It is difficult to imagine that there would be a significant difference in seed dispersal between the two regions, unless the difference in topography creates different dispersal patterns. Furthermore, we know that dispersal rate is fairly high in the clade; species have reached the Drakensberg at least five times, Madagascar twice, and within the last 1.3 Ma, Amsterdam Islands ~5,400 km away (Galley & Linder 2006a). Also, there are many lowland species of *Pentaschistis* which are very range restricted, and given the dispersal mechanism this is difficult to reconcile with the model of Latimer et al. (2005). Aside from these issues the model explains fairly range restricted species, e.g. to one mountain range. However we tested the importance of such species in explaining the turnover in the Cape, but they do not fully account for all of this;

partially restricted (non-widespread) species are a large part of this, and consequently also need to be explained.

Thirdly, it is possible that lower extinction rate in the Cape might have allowed range restricted species to persist there but not in the Drakensberg. The Cape was geomorphologically and climatically more stable than the Drakensberg in the late Pliocene and Pleistocene. The Pliocene uplift pushed parts of the Drakensberg up to ~850m but had little effect in the south western part of the continent (Partridge & Maud 1987). The coincidental interval of global cooling and aridification at this time (2.8 to 2.6 mya ago) would have culminated in more severe climatic change for the Drakensberg vegetation (Partridge 1998). However the effect is of this is difficult to reconcile with findings from other such systems, for example the Andes, where rapid radiation has been proposed following uplift 2 to 4 Mya ago (Hughes & Eastwood 2006). Additionally, and probably more importantly, is the relative stability of the Cape during Pleistocene glacial / inter-glacial cycling (Dynesius & Jansson 2000). This is thought to have lowered extinction rates in the Cape (Cowling et al. 1992; Goldblatt 1997; Dynesius & Jansson 2000) and would explain the high specialisation and small range sizes of many Cape *Pentaschistis* species in comparison to Drakensberg species (Dynesius & Jansson 2000). This might also explain the lower occupancy of habitats in the Drakensberg, if many species, especially the specialists, were removed from habitats in the Drakensberg, and these have since not had time to be re-occupied.

CONCLUSION

Explaining differences in diversity of a region brings together many disciplines (Qian & Ricklefs 2000; Ricklefs 2006) including ecology, phylogenetics, geology and physiology. The range of potential explanations for the incredible species richness of the Cape flora demonstrates that the region represents a microcosm of this challenge. We investigate the role of habitat heterogeneity to explain the difference in species turnover and richness of the *Pentaschistis* clade between the Cape and the Drakensberg. There are three main aspects of the habitat that we identify as important. Firstly, although the variety of habitats is similar in the two regions, the Cape has many more habitats that are occupied by *Pentaschistis*. We consider this measure more appropriate, and this suggests that heterogeneity of suitable habitat might indeed be important in influencing species richness. Explaining why species in

the Cape can occupy more of the habitats is the next challenge. The simplest explanation is that they have had more time to adapt to these often specialised niches. However we can exclude time as a factor since from the phylogeny it is evident that the genus has been in the Cape as long as in the Drakensberg. A higher speciation rate in the Cape might generate more specialist species. There is no additional evidence supporting this and we neither reject nor support this explanation. Lastly, it is possible that the Drakensberg was climatically less stable than the Cape during the Quaternary climatic fluctuations cycling. The accompanied extinction might have removed species from many habitats in the Drakensberg, which have since not been re-occupied. This also explains the second important difference between the *Pentaschistis* species from the two regions: more range restricted species persist in the Cape. Moderately range restricted species are additionally important in explaining the species turnover and also need to be explained. Whilst some species have range restricted niches, some are range restricted because they have distribution disequilibrium. Thirdly, we demonstrate higher overlap of species in our habitat units enabling greater landscape species coexistence in the Cape. This suggests there are parameters which are important niche dimensions for *Pentaschistis* which we did not include and these may be microhabitat parameters. Although it is likely that habitat characteristics alone do not explain all of the difference in species richness between the two areas, we demonstrate that they account for a substantial amount of the difference.

ACKNOWLEDGEMENTS

CG and HPL are grateful for funding from the University of Zurich and the Swiss Academy of Sciences.

APPENDIX 1

Table 1a. Standardized canonical discriminant functions for the Cape. Variables ordered by absolute size of correlation within function. * Largest absolute correlation between each variable and any discriminant function; (a) variable not used in the analysis. The first five functions explain 80.4% of the variance.

	Function													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
apan	.792(*)	.084	.332	-.055	-.065	-.376	.051	.100	.065	-.122	-.096	.143	.104	.188
solar_jun	.692(*)	.281	-.269	-.006	.164	-.460	-.194	.151	.079	-.141	-.095	.114	.059	.134
heat_1003	.612(*)	-.452	.020	-.096	.088	-.485	.052	.174	.063	-.005	-.188	.032	.081	.299
MAP	-.395(*)	-.385	-.201	-.393	.025	.339	.345	.245	.097	.022	.300	-.318	.043	.057
Tmin_jul(a)	.169	-.935(*)	.080	-.061	.145	.128	-.027	.021	-.126	.141	-.076	-.039	.006	.072
altitude	-.283	.906(*)	-.015	-.012	.029	.228	.082	-.076	-.015	-.067	.082	.092	-.028	-.114
heat_0409	.468	-.706(*)	-.031	.141	.275	-.229	-.122	.185	.143	.054	-.134	-.007	.058	.197
solar_jan	.282	.687(*)	.086	-.312	-.026	-.472	-.141	.173	.100	-.114	-.047	.157	.051	.133
frostdays	-.062	.642(*)	-.014	.133	-.093	-.087	.138	-.014	-.502	-.182	.423	.180	.105	.149
continenta	.372	.606(*)	-.048	-.124	-.088	-.577	.029	.193	.173	-.126	-.053	.157	.008	.159
Tmax_jan	.613	-.027	.007	-.208	.011	-.620(*)	.013	.262	.112	-.039	-.132	.165	.016	.262
soil_ferti	.145	.145	-.006	.169	.530	-.591(*)	.040	-.077	.044	-.253	-.066	.024	-.039	-.468
cvap	.415	.010	.044	-.125	.103	-.215	-.041	-.616(*)	.221	-.151	.308	.441	.055	.092
rain_season	.258	.131	.131	-.033	.008	.092	.065	-.010	-.099	.768(*)	.141	-.158	.424	-.246
rain_concentration	.251	.235	.252	-.094	.190	.069	.013	-.215	-.103	.333	.229	-.497	-.524(*)	.167

Table 1b. Standardized canonical discriminant functions for the Drakensberg. Variables ordered by absolute size of correlation within function. * Largest absolute correlation between each variable and any discriminant function; (a) variable not used in the analysis. The first five functions explain 96.1% of the variance.

	Function									
	1	2	3	4	5	6	7	8	9	10
Continenta	-.165	.592(*)	-.057	.163	-.466	.121	-.037	.008	.041	.287
MAP	.023	-.563(*)	.082	-.138	.265	.007	-.495	-.091	.090	.302
soil_ferti	-.118	.473	.691(*)	.126	.187	-.054	.116	-.185	-.082	.178
Altitude	-.057	-.363	-.189	-.036	.755(*)	-.245	.072	.192	-.139	-.053
heat_0409	.106	.033	.257	-.037	-.693(*)	.463	.113	-.071	.288	-.023
Apan	.140	.524	-.057	.089	-.659(*)	.322	-.084	-.010	.160	.155
heat_1003	.025	.373	.104	.153	-.645(*)	.461	-.081	-.068	.223	.019
solar_jan	-.168	.529	.077	.200	-.635(*)	.009	.046	.055	.004	.353
Tmax_jan	-.022	.466	.107	.151	-.634(*)	.388	-.021	-.043	.103	.185
Tmin_jul(a)	.163	.125	.267	.077	-.591(*)	.566	.007	-.089	.138	-.014
Frostdays	-.142	-.178	-.263	-.099	.589(*)	-.562	.265	.062	-.049	.300
solar_jun	.153	.199	-.291	.362	-.431(*)	.159	-.256	-.005	-.001	.270
Cvap	-.091	.355	.299	.089	-.410(*)	-.272	-.031	.372	.113	-.084
rain_season	-.058	-.038	-.322	.066	.381	.030	.160	-.696(*)	-.006	-.035
rain_concentration	.033	-.241	.029	.030	-.093	-.349	-.015	.023	.156	-.487(*)

Table 2a. Covariance matrix of the environmental variables for the Cape.

	altitude	APAN	Continent.	CVAP	frostdays	DD_Winter	DD_Summer	MAP	rain_conc	rain_season	soil_fertility	solar_jan	solar_jun	Tmax_jan	Tmin_jul
Altitude	1.0000														
APAN	-0.2619	1.0000													
Continent.	0.3550	0.6573	1.0000												
CVAP	-0.1155	0.5495	0.3510	1.0000											
frostdays	0.7170	0.0286	0.4523	0.0764	1.0000										
DD_winter	-0.9032	0.4617	-0.1221	0.2354	-0.6495	1.0000									
DD_summer	-0.7605	0.7603	0.2856	0.3855	-0.4152	0.8728	1.0000								
MAP	-0.1821	-0.5957	-0.5922	-0.4403	-0.2921	-0.0353	-0.2129	1.0000							
rain_conc	0.1536	0.2259	0.1476	0.1183	0.1248	-0.0774	0.0156	-0.1427	1.0000						
rain_season	0.0371	0.2231	0.0622	0.0308	0.0436	0.0154	0.0680	-0.1166	0.3268	1.0000					
soil_fertility	0.0467	0.2853	0.4038	0.3001	0.0780	0.1600	0.2253	-0.4531	0.0084	-0.0700	1.0000				
solar_jan	0.4792	0.5703	0.9483	0.3095	0.4964	-0.2683	0.1349	-0.5589	0.1965	0.0970	0.3371	1.0000			
solar_jun	-0.0661	0.7845	0.7947	0.4905	0.1457	0.3720	0.6262	-0.6244	0.1354	0.1115	0.4806	0.6906	1.0000		
Tmax_jan	-0.3824	0.8814	0.7059	0.4597	-0.0855	0.5696	0.8705	-0.4327	0.0870	0.0838	0.3434	0.5890	0.8451	1.0000	
Tmin_jul	-0.9211	0.0542	-0.6205	0.0168	-0.7289	0.8017	0.5634	0.3513	-0.1106	0.0056	-0.1859	-0.6775	-0.1786	0.1173	1.0000

Table 2b. Covariance matrix of the environmental variables for the Drakensberg.

	altitude	APAN	Continent.	CVAP	frostdays	DD_Winter	DD_Summer	MAP	rain_conc	rain_season	soil_fertility	solar_jan	solar_jun	Tmax_jan	Tmin_jul
altitude	1.0000														
APAN	-0.8804	1.0000													
Continent.	-0.6830	0.7362	1.0000												
CVAP	-0.5111	0.4215	0.5043	1.0000											
frostdays	0.7024	-0.6249	-0.2200	-0.2065	1.0000										
DD_Winter	-0.8002	0.6556	0.3730	0.3005	-0.7651	1.0000									
DD_Summer	-0.9409	0.8963	0.7488	0.4602	-0.7318	0.8217	1.0000								
MAP	0.5101	-0.5066	-0.5859	-0.5171	0.1751	-0.2751	-0.5185	1.0000							
Rain_conc	0.1350	-0.1168	-0.2162	-0.1129	0.1116	-0.1381	-0.1923	0.1696	1.0000						
rain_season	0.3373	-0.2815	-0.1573	-0.4020	0.3315	-0.3074	-0.2953	0.1742	-0.0543	1.0000					
soil_fertility	-0.2492	0.1377	0.4596	0.2883	-0.0256	0.0722	0.2339	-0.3232	-0.1736	-0.1055	1.0000				
solar_jan	-0.6132	0.6612	0.8671	0.4976	-0.3005	0.3669	0.6303	-0.4346	-0.1846	-0.2172	0.4343	1.0000			
solar_jun	-0.5509	0.6981	0.6136	0.1450	-0.3556	0.4610	0.6426	-0.2350	-0.1273	-0.0855	-0.0401	0.4502	1.0000		
Tmax_jan	-0.9174	0.9171	0.8526	0.5061	-0.5913	0.6869	0.9525	-0.5443	-0.1855	-0.2755	0.3290	0.7600	0.6103	1.0000	
Tmin_jul	-0.7898	0.7182	0.2464	0.2654	-0.8030	0.7760	0.7669	-0.2274	-0.0554	-0.3009	-0.0032	0.2520	0.3128	0.7166	1.0000

APPENDIX 2

Table 1. Species lists for the four sub-regions of the Cape. Species unique to that sub-region are shown in bold.

Cape Peninsula	Cederberg	Langeberg	Hexrivierberg
Pentameris longiglumis ssp. longiglumis	Pentameris distichophylla	Pentameris macrocalycina	Pentameris distichophylla
Pentameris macrocalycina	Pentameris macrocalycina	**Pentameris thuarii**	Pentameris macrocalycina
Pentaschistis acinosa	Pentaschistis airoides ssp. airoides	**Pentameris uniflora**	**Pentameris oreophila**
Pentaschistis airoides ssp. airoides	Pentaschistis alticola	Pentaschistis acinosa	Pentameris thuarii
Pentaschistis ampla	Pentaschistis ampla	Pentaschistis ampla	Pentaschistis airoides ssp. airoides
Pentaschistis argentea	Pentaschistis aristidoides	Pentaschistis argentea	Pentaschistis alticola
Pentaschistis aristidoides	**Pentaschistis capillaris**	Pentaschistis aristidoides	Pentaschistis ampla
Pentaschistis aspera	Pentaschistis colorata	Pentaschistis aurea ssp. aurea	Pentaschistis aspera
Pentaschistis barbata	Pentaschistis curvifolia	Pentaschistis capensis	Pentaschistis aurea ssp. aurea
Pentaschistis capensis	Pentaschistis densifolia	**Pentaschistis cirrhulosa**	Pentaschistis capensis
Pentaschistis colorata	Pentaschistis eriostoma	Pentaschistis colorata	Pentaschistis colorata
Pentaschistis curvifolia	Pentaschistis glandulosa	Pentaschistis curvifolia	Pentaschistis curvifolia
Pentaschistis ecklonii	Pentaschistis malouinensis	Pentaschistis malouinensis	Pentaschistis densifolia
Pentaschistis glandulosa	Pentaschistis pallida	Pentaschistis pallida	Pentaschistis eriostoma
Pentaschistis malouinensis	**Pentaschistis pungens**	Pentaschistis pyrophila	Pentaschistis glandulosa
Pentaschistis pallescens	Pentaschistis pusilla	Pentaschistis rigidissima	**Pentaschistis horrida**
Pentaschistis pallida	Pentaschistis pyrophila	Pentaschistis tortuosa	Pentaschistis malouinensis
Pentaschistis papillosa	**Pentaschistis reflexa**		**Pentaschistis montana**
Pentaschistis pusilla	Pentaschistis rigidissima		Pentaschistis pallescens
Pentaschistis tortuosa	Pentaschistis rosea ssp. purpurascens		Pentaschistis pallida
	Pentaschistis rupestris		**Pentaschistis pseudopallescens**
	Pentaschistis triseta		Pentaschistis pyrophila
	Pentaschistis velutina		Pentaschistis rigidissima
	Pentaschistis veneta		Pentaschistis rosea ssp. purpurascens
			Pentaschistis viscidula
			Pentaschistis veneta

Table 2. Species lists for the five sub-regions of the Drakensberg. Species unique to that sub-region are shown in bold.

Cathedral Peak	Cathkin Peak	Mount aux Sources	Naudes Nek to Lundeans Pass	Sani Pass to Bushmans Nek
Pentaschistis aurea ssp. aurea	Pentaschistis aurea ssp. aurea	Pentaschistis aurea ssp. aurea	Pentaschistis aurea ssp. aurea	Pentaschistis aurea ssp. aurea
Pentaschistis exserta	Pentaschistis exserta	Pentaschistis exserta	Pentaschistis exserta	Pentaschistis exserta
Pentaschistis galpinii	Pentaschistis oreodoxa	Pentaschistis galpinii	Pentaschistis galpinii	Pentaschistis galpinii
Pentaschistis oreodoxa	Pentaschistis setifolia	Pentaschistis oreodoxa	Pentaschistis oreodoxa	Pentaschistis oreodoxa
Pentaschistis setifolia		Pentaschistis setifolia	Pentaschistis setifolia	**Pentaschistis praecox**
Pentaschistis tysonii		Pentaschistis tysonii	Pentaschistis tysonii	Pentaschistis setifolia
				Pentaschistis tysonii

APPENDIX 3

	environmental variable	Drakensberg	Cape
altitude	**mean**	***307.55**	**226.76**
	st dev	188.58	199.88
	range	1315	1452
APAN	**mean**	**11.91**	***134.42**
	st dev	79.43	136.23
	range	624.08	1186.7
continentality	**mean**	***4.18**	**2.01**
	st dev	1.96	1.11
	range	17	6.98
CVAP	**mean**	**3.06**	***3.68**
	st dev	2.33	3.17
	range	19	25
degree days (Winter)	**mean**	**71.50**	***160.87**
	st dev	61.04	136.66
	range	548	763
degree days (Summer)	**mean**	***310.99**	**215.78**
	st dev	237.16	209.53
	range	1554.9	1286
frost days	**mean**	***42.904**	**5.25**
	st dev	40.563	7.71
	range	254	69
MAP	**mean**	**75.24**	***84.00**
	st dev	75.24	98.87
	range	1095.00	1822.2
rain concentration	**mean**	**0.42**	***1.46**
	st dev	0.51	1.200
	range	2	10
rain season	mean	0.07	*0.12
	st dev	0.25	0.72
	range	2	5
soil fertility	**mean**	**0.17**	***0.42**
	st dev	0.57	1.05
	range	3.8	4.9
solar radiation (Jan)	**mean**	***2.32**	**0.79**
	st dev	2.20	0.833
	range	-0.12	0.04
solar radiation (Jul)	**mean**	***2.30**	**0.33**
	st dev	1.41	0.34
	range	8	2.5
T-max (Jan)	**mean**	***2.39**	**1.25**
	st dev	1.75	1.36
	range	10.00	9.00
T-max (Jul)	**mean**	***1.94**	**0.78**
	st dev	1.62	0.89
	range	10.00	6.00

Table 1. List of the 'roughness' statistics for each environmental variable, for the Drakensberg and the Cape. Each minute by minute cell was considered, alongside its eight neighbours. For a given environmental variable, the range of values in the neighbourhood of nine cells was calculated. The distributions of these range values were calculated for the Cape and the Drakensberg. The mean, standard deviation and range of these distributions are given. An asterisk is shown by the mean which is the highest between the two regions. 'Roughness' measures the local variation in the environmental variables. For definitions of the variables, see main text.

APPENDIX 4

	environmental variable	Drakensberg	CFR
altitude	mean	1960.2	506.64
	st dev	***433.28**	**362.4**
	range	2,264	2,073
APAN	mean	1709	2142.2
	st dev	**207.53**	***291.38**
	range	1,209	2,257
continentality	mean	24.87	23.9
	st dev	**2.47**	***3.40**
	range	15	18
CVAP	mean	25.36	32.90
	st dev	**3.56**	***5.23**
	range	19	25
degree days (Winter)	mean	163.93	651.12
	st dev	**125.4**	***245.7**
	range	736	1,310
degree days (Summer)	mean	1096	1782.6
	st dev	***496.12**	**269.79**
	range	1,919	2,053
frost days	mean	69.17	12.77
	st dev	***41.58**	**12.80**
	range	259	80
MAP	mean	719.38	368.89
	st dev	**147.49**	***205.9**
	range	1,550	3,171
rain season	mean	4.26	2.07
	st dev	**0.48**	***1.42**
	range	3	5
rain concentration	mean	50.635	31.94
	st dev	**4.25**	***19.90**
	range	20	69
soil fertility	mean	3.80	3.22
	st dev	***1.05**	**1.42**
	range	3.8	6.4
solar radiation (Jan)	mean	23.91	32.43
	st dev	***3.67**	**3.09**
	range	20	16
solar radiation (Jul)	mean	15.11	12.66
	st dev	***1.4568**	**0.90**
	range	8	6
T-max (Jan)	mean	23.91	28.47
	st dev	***3.67**	**2.32**
	range	20	15
T-max (Jul)	mean	-0.89	4.57
	st dev	***2.10**	**1.89**
	range	16	12

Table 1. List of the environmental variables. Each minute by minute cell was considered, and its value for each variable was recorded. The mean, standard deviation (st dev) and the range of the values that occur in the CFR and the Drakensberg are given. An asterisk is given by the highest standard deviation for every variable. The standard deviation indicates the relative length of the environmental gradient in the CFR and in the Drakensberg. For definitions of the variables, see main text.

REFERENCES CITED

ArcGIS. 1999-2002: ESRI Inc.

Barker, N. P. 1993 A biosystematic study of *Pentameris* (Arundineae, Poaceae). *Bothalia* **23**, 25-47.

Bennet, K. D. 1988 Holocene geographic spread and population expansion of *Fagus grandifolia* in Ontario, Canada. *Journal of Ecology* **76**, 547-557.

Broennimann, O., Thuiller, W. & Hughes, G. 2006 Do geographic distribution, niche property and life form explain plants' vulnerability to glocal change? *Global Change Biology* **12**, 1079-1093.

Carbutt, C. & Edwards, T. 2002 Cape elements on high-altitude corridors and edaphic islands: historical aspects and preliminary phytogeography. *Systematics and Geography of Plants* **71**, 1033-1061.

Cody, M. L. 1986 Diversity, rarity, and conservation in Mediterranean-climate regions. In *Conservation science: the science of scarcity and diversity* (ed. M. E. Soulé), pp. 123-152. Sunderland, Massachusetts, USA: Sinauer Associates Inc.

Cowling, R. M. 1983 Phytochorology and vegetation history in the south-eastern Cape, South Africa. *Journal of Biogeography* **10**, 393-419.

Cowling, R. M. 1987 Fire and its role in coexistence and speciation in Gondwanan shrublands. *South African Journal of Science* **83**, 106-112.

Cowling, R. M. 1990 Diversity components in a species-rich area of the Cape Floristic Region. *Journal of Vegetation Science* **1**, 699-710.

Cowling, R. M. & Heijnis, R. H. M. 2001 The identification of Broad Habitat Units as biodiversity entities for systematic conservation planning in the Cape Floristic Region. *South African Journal of Botany* **67**, 15-38.

Cowling, R. M. & Holmes, M. A. 1992 Endemism and speciation in a lowland flora from the Cape Floristic Region. *Biological Journal of the Linnean Society* **47**, 367-383.

Cowling, R. M., Holmes, P. M. & Rebelo, A. G. 1992 Plant diversity and endemism. In *The ecology of fynbos. Nutrients, fire and biodiversity* (ed. R. M. Cowling), pp. 62-112. Cape Town: Oxford University Press.

Cowling, R. M. & Lombard, A. T. 2002 Hetergeneity, speciation/extinction history and climate: explaining regional plant diversity patterns in the Cape Floristic Region. *Diversity and Distributions* **8**, 163-179.

Cowling, R. M., Rundel, P. W., Lamont, B. B., Arroyo, M. K. & Arianoutsou, M. 1996 Plant diversity in Mediterranean-climate regions. *Trends in Ecology and Evolution* **11**, 362-366.

Davidse, D. 1988 A revision of the genus *Prionanthium* (Poaceae : Arunidnoideae). *Bothalia* **18**, 143-153.

Dynesius, M. & Jansson, R. 2000 Evolutionary consequences of changes in species' geographical distributions driven by Milankovitch climate oscillations. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 9115-9120.

Galley, C. & Linder, H. P. 2006a The Cape element in the Afrotemperate flora: from Cape to Cairo? *Proceedings of the Royal Society of London Series B Biological Sciences* **274:** 535-543.

Galley, C. & Linder, H. P. 2006b New species and taxanomic changes within *Pentaschistis* (Danthonioideae, Poaceae) from the Western Cape Province, South Africa. *Bothalia* **36**, 157-162.

Galley, C. & Linder, H. P. 2007 The phylogeny of the *Pentaschistis* clade (Danthonioideae, Poaceae) based on cpDNA, and the evolution and loss of complex characters. *Evolution* 2007 **61**, 864-884.

Goldblatt, P. 1978 An analysis of the flora of Southern Africa: its characteristics, relationships, and origins. *Annals of the Missouri Botanical Garden* **65**, 369-436.
Goldblatt, P. 1997 Floristic diversity in the Cape Flora of South Africa. *Biodiversity and Conservation* **6**, 359-377.
Goldblatt, P. & Manning, J. C. 2002 Plant diversity of the Cape region of Southern Africa. *Annals of the Missouri Botanical Garden* **89**, 281-302.
Guisan, A. & Thuiller, W. 2005 Predicting species distribution: offering more than simple habitat models. *Ecology Letters* **8**, 993-1009.
Hilliard, O. M. & Burtt, B. M. 1987 *The botany of the Southern Natal Drakensberg*. Annals of Kirstenbosch Botanic Gardens. Pretoria: NBI.
Hughes, C. & Eastwood, R. 2006 Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 10334-10339.
Huntley, B. 2006 Reconstructing past environments from the Quaternary palaeovegetation record. *Proceedings of the Royal Irish Academy* **101B**, 3-18.
Johnson, S. D. & Steiner, K. E. 2003 Specialized pollination systems in southern Africa. *South African Journal of Science* **99**, 345-348.
Kruger, F. J. & Taylor, H. C. 1979 Plant species diversity in Cape fynbos: gamma and delta diversity. *Vegetacio* **41**, 85-93.
Latimer, A. M., Silander, J. A. J. & Cowling, R. M. 2005 Neutral ecological theory reveals isolation and rapid speciation in a biodiversity hotspot. *Science* **309**, 1722-1725.
Leibold, M. 1995 The niche concept revisited: mechanistic models and community context. *Ecology* **76**, 1371-1382.
Linder, H. P. 1985 Gene flow, speciation, and species diversity patterns in a species-rich area: the Cape Flora. In *Species and speciation*, vol. 4 (ed. E. S. Vrba), pp. 53-57: Transvaal Museum Monograph.
Linder, H. P. 1991 Environmental correlates of patterns of species richness in the south-western Cape Province of South Africa. *Journal of Biogeography* **18**, 509-518.
Linder, H. P. 2003 The radiation of the Cape flora, southern Africa. *Biological Reviews* **78**, 597-638.
Linder, H. P. 2005 A protocol for the systematic documentation of the ecology of Cape plants. *South African Journal of Botany* **71**, 201-210.
Linder, H. P. & Ellis, R. P. 1990 A revision of *Pentaschistis* (Arundineae: Poaceae). *Contributions from the Bolus Herbarium* **12**.
Marquet, P. A., Keyner, J. E. & Cofré, H. 2003 Breaking the stick in space: of niche models, metacommunities and patterns in the relative abundance of species. In *Macroecology: concepts and consequences* (ed. T. A. Blackburn & K. J. Gaston), pp. 64-84. Mainden, USA; Oxford, UK; Victoria, Australia: Blackwell Science Ltd.
McDonald, D. J. & Cowling, R. M. 1995 Towards a profile of an endemic mountain fynbos flora: implications for conservation. *Biological Conservation* **72**, 1-12.
Mutke, J. & Barthlott, W. 2005 Patterns of vascular plant diversity at continental to global scales. In *Plant diversity and complexity patterns - local, regional and global dimensions* (ed. I. Friis & H. Balslev), pp. 521-537. Copenhagen, Denmark: The Royal Danish Academy of Sciences and Letters.
NTSYSpc. 2000-2003: Applied Biostatistics.
Oliver, E. G. H., Linder, H. P. & Rourke, J. P. 1983 Geographical distribution of present-day Cape taxa and their phytogeographical significance. *Bothalia* **14**, 427-440.
Partridge, T. C. 1998 Of diamonds, dinosaurs and diatrophism: 150 million years of landscape evolution in southern Africa. *South African Journal of Geology* **101**, 167-184.

Partridge, T. C. & Maud, R. R. 1987 Geomorphic evolution of Southern Africa since the Mesozoic. *South African Journal of Geology* **90**, 179-208.
Phillips, S. M. 1994 Variation in the *Pentaschistis pictigluma* complex (Gramineae). In *XIIIth Plenary Meeting AETFAT, Malawi*, vol. 1 (ed. J. H. Seyani & A. C. Chikuni), pp. 359-372. Zomba, Malawi: National Herbarium and Botanic Gardens of Malawi.
Phillips, S. M. 1995 A new species of *Pentaschistis* (Gramineae) from Ethiopia. *Kew Bulletin* **50**, 615-617.
Qian, H. & Ricklefs, R. E. 2000 Large-scale processes and the Asian bias in species diversity of temperate plants. *Nature* **407**, 180-182.
Ricklefs, R. E. 2006 Evolutionary diversification and the origin of diversity-environment relationship. *Ecology* **87**, S3-S13.
Rosenzweig, M. L. 1995 *Species diversity in space and time*. Cambridge, UK: Cambridge University Press.
Savolainen, V. & Forest, F. 2005 Species-level phylogenetics from continental biodiversity hotspots. In *Plant species-level systematics: new perspectives on pattern and process* (ed. F. T. Bakker, L. W. Chatrou, B. Gravendeel & P. B. Pelser). Ruggell, Liechtenstein: A.R.G. Gantner Verlag.
Schluter, D. & Ricklefs, R. E. 1993 Species diversity: An introduction to the problem. In *Species diversity in ecological communities; historical and geographical perspectives* (ed. R. E. Ricklefs & D. Schluter). Chicago and London: The University of Chicago Press.
Schulze, R. E. 1997 South African Atlas of Agrohydrology and Climatology, Report TT82/96. Pretoria: Water Research Commission.
Simmons, M. T. & Cowling, R. M. 1996 Why is the Cape Peninsula so rich in plant species? An analysis of the independent diversity components. *Biodiversity and Conservation* **5**, 551-573.
SPSS. 2003: SPSS inc.
Stockwell, D. R. B. & Peterson, A. T. 2002 Effects of sample size on accuracy of species distribution models. *Ecological Modelling* **148**, 1-13.
Swofford, D. L. 2002 PAUP* Phlyogenetic analysis using parsimony (and other methods) 4.0 beta version. Florida State University.
Szabo, P. & Meszéna, G. 2006 Limiting similarity revisited. *OIKOS* **112**, 612-619.
Tinner, W. & Lotter, A. F. 2006 Holocene expansions of *Fagus silvatic* and *Abies alba* in Central Europe: where are we after eight decades of debate? *Quaternary Science Reviews* **25**, 526-549.
Whittaker, R. H. 1977 Species diversity in land communities. *Evolutionary Biology* **10**.
Whittaker, R. J., Willis, K. J. & Field, R. 2001 Scale and species richness: towards a general, hierarchical theory of species diversity. *Journal of Biogeography* **28**.
Wilson, M. V. & Shmida, A. 1984 Measuring beta diversity with presence-absence data. *Journal of Ecology* **72**, 1055-1064.

New species and taxonomic changes within *Pentaschistis* (Danthonioideae, Poaceae) from Western Cape, South Africa

CHLOE GALLEY AND H. PETER LINDER

Institute of Systematic Botany, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland

Bothalia 2006, **36**: 157-162

ABSTRACT

Three new species of *Pentaschistis* (Nees) Stapf are described from the Cape Floristic Region, *P. trifida*, *P. clavata* and *P. horrida*. The former has been collected from inland ranges of the Cape Fold Belt, from the Cederberg to the Groot Swartberg, the latter two each from single sites in the Koue Bokkeveld: the former on the wetter western border, and the latter on the Baviaansberg. Additionally we resurrect *Pentaschistis juncifolia* Stapf, a species from the coastal plains (Hardeveld) between Bredasdorp and Riversdale, which had been included in *Pentaschistis* eriostoma (Nees) Stapf.

Keywords: Cape Floristic Region, Danthonioideae, fynbos, new species, *Pentaschistis* (Nees) Stapf, Poaceae, South Africa

INTRODUCTION

The remarkable fieldwork of Ms Esterhuysen resulted in the description of many new species of *Pentaschistis* (Nees) Stapf (Linder & Ellis 1990), but ongoing fieldwork and taxonomic research on the grasses of the Cape Floristic Region (CFR) (Goldblatt 1978) is resulting in the occasional discovery of new grass species.

Pentaschistis comprises 68 recognized species and is the most species-rich grass genus in the CFR (Goldblatt & Manning 2000; Linder 1989). The majority of species are endemic to or centred in this region. Additionally there are eleven species in the Drakensberg region, seven species in the tropical east African mountains from Malawi to Ethiopia, one species on Mt Cameroon, three species in Madagascar, one endemic species in the Imatong Mountains, Sudan, and one endemic species on St. Paul and Amsterdam Islands in the South Indian Ocean. A few of the more drought-tolerant species occur in the drier northwest of South Africa into the Greater Cape Floristic Region (Jürgens 1991).

Despite the recent revision of the genus (Linder & Ellis 1990), there are still a number of taxonomic problems remaining. Some species show enormous variation over habitat and geographical range, such as the *P. pictigluma* complex in eastern Africa (Phillips 1994), and the *P. pallida* complex in the CFR. There have also been a steady rate of discovery and descriptions of new species in the genus over the past 15 years (Linder & Ellis 1990; Phillips 1986; Phillips 1995). Here we describe a further three new species. *Pentaschistis trifida* was found by the first author on a recent collecting trip; *P. clavata* was found by the late Mr Hugh Taylor and recognized as new by Mrs Lynn Fish of PRE. *P. horrida* had already been recognized as distinctive by Dr. Roger Ellis and the second author (Linder & Ellis 1990), but they included it under *P. rigidissima*. After extensive fieldwork we were convinced that it is indeed a distinct species, for the reasons given below. We also resurrect *Pentaschistis juncifolia* Stapf to species level following observation of differences in ecology and habit between this taxon and *Pentaschistis eriostoma* (Nees) Stapf.

SPECIES DESCRIPTIONS

Pentaschistis trifida C.A. Galley, sp. nov., Pentaschistis trisetae (Thunb.) Stapf similis sed spiculis unifloris, glantibus ellipticis, dimensionibus spiculorum parvioribus (gluma infirior

4.5---5.5 mm longa, non15---18 mm, lemma 2.4---4.8 mm longa, non 6---8 mm, arista lemmatis 18---24 mm longa, non 25---35 mm), glumis omnino parce puberulis, lemmatibus inter venas dense villosis, paleis glabris, et ramis floriferis trifurcatis.

TYPE.—WESTERN CAPE, 3319 (Ceres): Baviaansberg, north of the Hex River Mountains, 1 050 m, 33012 '14.6"S, 19037'04.5" E; (–BA), 11 Nov. 2004, C. A. Galley 577 (ZH, holo.; BOL, K, NBG, PRE).

Perennial; single or few stems. Culms 80---200 mm tall; nodes glabrous; basal sheaths white shiny, persistent; prophylls truncate, often bilobed, keels remaining parallel to apex; innovation buds intravaginal; with elliptically shaped, linear-type, multicellular glands present. Leaves basal; sheaths red-purple, sparsely puberulous; sheath mouth glabrous; blades puberulous; ligules 0.25 mm long fringe of hairs; blades 20---50 mm x 0.5---1 mm, rolled, the apex acute, margins scaberulous; old blades persisting entire. Inflorescences widely paniculate, open, with 15---35 spikelets, 50---75 x 40---60 mm; pedicels mostly erect, longer or shorter than spikelets; inflorescence branches longer than spikelets, glabrous, with multicellular glands; nodes glabrous. Spikelets 1-flowered, 4.5---5.5 mm long. Lower glume longer than floret, acute, 1- veined, 4.5---5.5 mm long, pale yellow with purplish base and green tip; margins same texture as body of glume; puberulous. Upper glume similar to lower glume but slightly shorter. Lemma 2.4---2.8 mm long, hairs villous, scattered on back of lemma between veins, veins 5; apex lobed, lobes acute, 0.5 mm long, lobe setae 6---8 mm long; lemma awn geniculate, 18---24 mm long, column twisted, 5.5 mm long; veins 5. Palea glabrous, linear to lorate, 3 x 0.4 mm acute, as long as lemma, keels parallel, glabrous. Callus to 0.8 mm long, densely hairy with short hairs at base and long hairs at top. Anthers 0.9---1.2 mm long. Ovary stalked, turbinate; styles two. Flowering time late October to early November. Figure 1 A–H.

Etymology: The specific epithet ‘trifida’ is named after the unique trichotomous branching pattern in parts of the inflorescence; in the rest of the genus the branches are paired.

Diagnostic characteristics: *Pentaschistis trifida* resembles *P. triseta* (Thunb.) Stapf by having reduced leaves and a long lemma awn in relation to the spikelet. It resembles *P. pusilla* (Nees) H.P.Linder and *P. clavata* (described here) by the single floret per spikelet, and *P. caulescens* H.P.Linder as both species occupy disturbed habitats and have reduced leaves

with red / purple leaf sheaths. However, it can be easily distinguished from these four species by the characteristics shown in Table 1. Unique to this new species is the elongated callus, the trifurcating inflorescence branches and the elliptically shaped linear-type glands on the inflorescence branches (Figure 1D), which contrast clearly with the purple branches.

Distribution and habitat: this species was collected from a shaded disturbed (pathside) habitat of deep, sandy soil derived from Table Mountain in a fairly arid area on the eastern side of the Baviaansberg (Figure 2). Although this is perhaps a more opportunistic habitat typical of an annual species (e.g. *P. airoides* (Nees) Stapf subsp. *airoides* habitat) this plant is a perennial; the highly reduced leaves suggest that the water requirement of this species is, however, probably low. Although locally common, this was the only population found in the vicinity.

Table 1. Comparison between *Pentaschistis trifida* and other species

	P. trifida	*P. triseta*	*P. caulescens*	*P. pusilla*	*P. clavata*
lifeform	perennial	annual	perennial	perennial	perennial
habit	caespitose	n/a	tangled	forming mats	cushion
leaves	highly reduced	not highly reduced	not highly reduced	not highly reduced	not highly reduced
inflorescence size	50---75 mm x 40---60 mm	70---100 mm x 30---90 mm	30---50 mm x 20---40 mm	20 x 15 mm	20---35 x 15---35 mm
florets per spiklet	one	two	two	one	one
lemma awn	present	present	present	absent	absent
lemma length	2.4---4.8 mm	6---8 mm	3 mm	1.5---3.0 mm	2.3 mm
glume length	4.5---5.5 mm	15---18 mm	8---12 mm	2.5---3.0 mm	2.2---2.5 mm
glume indumentum	sparsely puberulous all over	tuberculate hairs along margins	glabrous	glabrous	glabrous

Pentaschistis clavata C.A. Galley, sp. ab P. pusilla (Nees) H.P.Linder differt pilis clavatis lemmatis, lamina foliorum setosa, involuta.

TYPE.—WESTERN CAPE, 3219 (Wuppertal): Koue Bokkeveld Mountains south of Hexberg, on the Farm De Boom, 1 212m, 32044'32.1"S, 19011'35.2"E, (–BA), 7 Nov. 2004, C.A. Galley 567 (ZH, holo.; BOL, E, G, K, MO, NBG, NSW, NY, PRE, S, UPS, W)

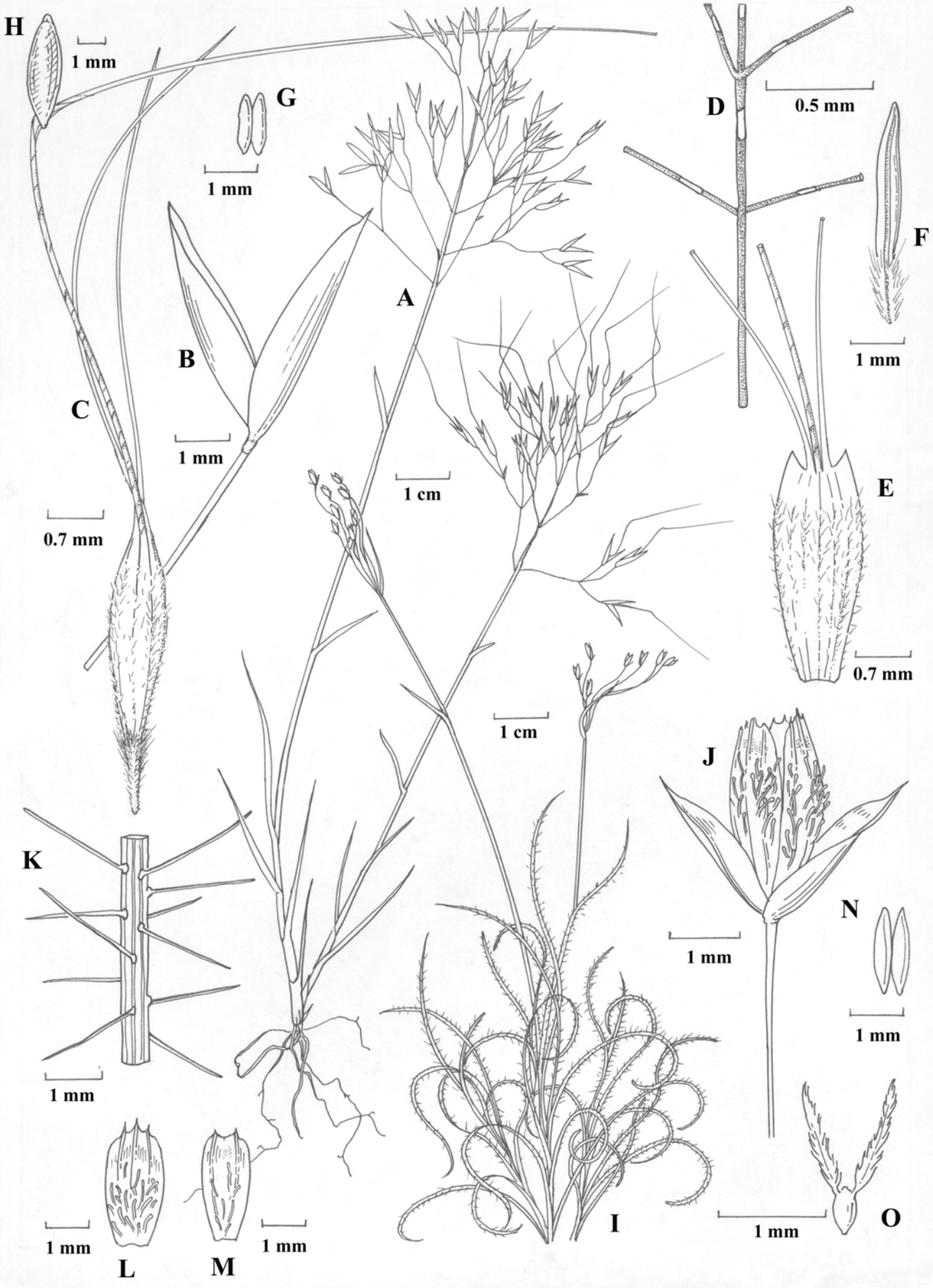
Plants perennial, forming neat rounded cushions, up to 100 mm in diam.; without multicellular glands. Culms 80---200 mm tall; nodes puberulous; basal sheaths white shiny,

persistent; prophylls truncate, often bilobed, keels remaining parallel to apex; innovation buds intravaginal. Leaves cauline; sheaths glabrous or with sparse, 2 mm long tubercle-based hairs; sheath mouth with a ring of 2 mm long, stiff bristles; ligules 0.2 mm long fringes of hair; blades 40---50 x 1 mm, expanded at base, with sparse, scattered 2 mm long tubercle-based bristles; apex keeled, acute; margins smooth; old blades persisting entire. Inflorescences widely paniculate, open, with 10---20 spikelets, 20---35 x 15---35 mm; pedicels mostly erect, longer than spikelets; inflorescence branches longer than spikelets, puberulous; nodes villous with long erect hairs. Spikelets 1-flowered, 2.5 mm long. Lower glume as long as or shorter than floret, acute, 1-veined, 2.0---2.5 mm long, straw-coloured with purplish base; margins same texture as body of glumes, glabrous. Upper glume similar to lower glume, but somewhat shorter and narrower. Lemma 2.2---2.5 mm long, clavate hairs scattered between veins on back, veins 7, not anastomosing, apex finely tridentate. Palea glabrous or with few clavate hairs found between the keels, linear to lorate, truncate, 2.2 x 0.5 mm; as long as lemma; keels parallel, glabrous. Lodicules without microhairs or bristles; obtriangular, 3- veined. Anthers 1.6---1.9 mm long. Ovary stalked, turbinate; styles two. Figure 1 I–O. Flowering time November and December.

Etymology: the species epithet ‘clavata’ is named after the clavate lemma hairs.

Diagnostic characteristics: *Pentaschistis clavata* resembles *P. pusilla* in the single-flowered spikelet. This is generally a rare feature in the Danthonioideae, and within *Pentaschistis* is only found in two species, *P. pusilla* (Linder & Ellis 1990) and *P. trifida* described in this paper. *P. clavata* has a finely tridentate lemma apex, similar to that of *P. pusilla*, and in addition the generally soft, orthophyllous leaves and weakly perennial habit are reminiscent of that found in *P. pusilla*. However, there are several convincing differences (see Table 2). The new species has scattered, stout, clavate hairs on the lemma back, the only known case of clavate hairs in *Pentaschistis*. Clavate hairs occur in several other genera of the Danthonioideae, such as *Karroochloa* (Conert & Türper 1969), *Schismus* (Conert & Türper 1974) and *Tribolium* (Linder & Davidse 1997), but these genera are all rather distantly related to *Pentaschistis*.

Figure 1. (following page) *Pentaschistis trifida*, Galley 577: A, whole plant; B, glume pair; C, floret showing long callus; D, inflorescence stem showing glands; E, lemma; F, palea with callus; G, anther; H, caryopsis. *Pentaschistis clavata*, Galley 567: I, whole plant; J, spikelet showing clavate hairs; K, leaf showing tubercle-based hairs; L, lemma; M, palea; N. anther; O, ovary and styles. Artist: Claire Linder-Smith.


H
1 mm
G
1 mm
D
0.5 mm
F
1 mm
A
B
1 mm
C
0.7 mm
1 cm
E
0.7 mm
1 cm
J
K
1 mm
1 mm
N
1 mm
I
1 mm
O
1 mm
L
1 mm
M
1 mm

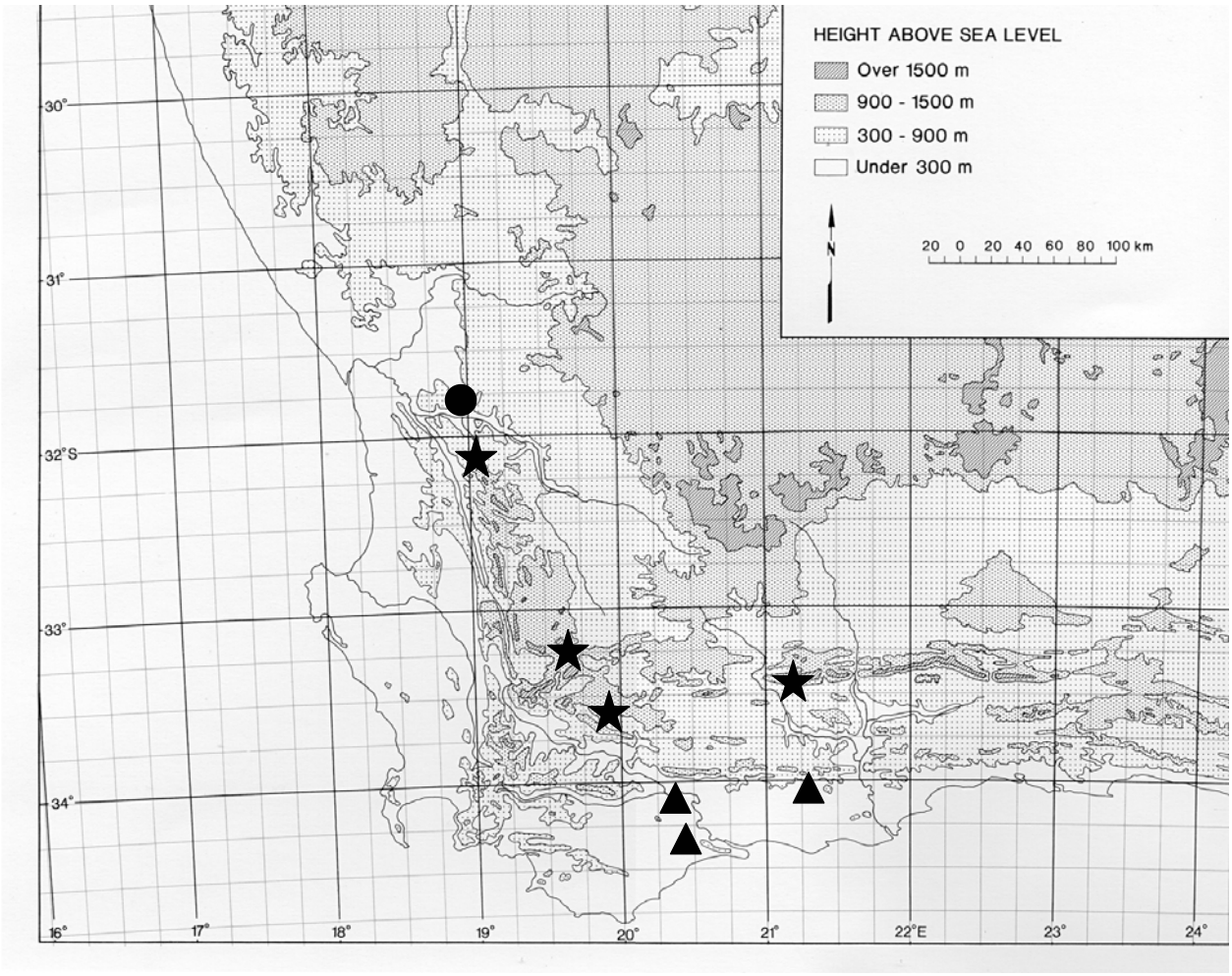


Figure 2. Distribution map of *Pentaschistis clavata*, (circle); *P. horrida*, (star); *P. trifida*, (diamond); and *P. juncifolia*, (triangle).

Distribution and habitat: this new species is known from a single locality above De Boom in the Koue Bokkeveld, at an altitude of 1 270 m (Figure 2). The species was first collected by Mr Hugh Taylor in 1989, describing the habitat as 'Streambank in moist soil with moss'. Further investigations in 1998 and 2005 showed that the species is quite common in the area, occurring on damp sand derived from Table Mountain sandstones as well as streambanks in wet moss. The habitat therefore differs slightly from that of *P. pusilla*. It is possible that the species is much more widespread—these mountains are still poorly explored, and more populations may be found in similar habitats.

Other specimens examined: WESTERN CAPE.—3219 (Wuppertal): south of Hexberg, Koue Bokkeveld Mountains, 1 270m, (–CC), H.C. Taylor 12095 (PRE).

Table 2. comparison between *Pentaschistis clavata* and *P. pusilla*

	P. clavata	*P. pusilla*
leaf indumentum	with scattered bristles	glabrous
leaf insertion	on aerial shoots	basal
inflorescence indumentum	nodes villous	glabrous
lemma indumentum (abaxial)	scattered, stout clavata hairs	fine tapering hairs (some collections from the eastern range are almost glabrous)

Pentaschistis horrida C.A. Galley, sp. nov., *Pentaschistis* rigidissimae Pilger ex Linder similis sed differt longitudine majore culmorum et foliorum, ramificatione caulium, indumento superficiei superior foliorum et dimensione majore anthera, 2.1---2.8 mm (non 1.4---1.8 mm).

TYPE.—WESTERN CAPE, 3319 (Ceres): Baviaansberg, north of the Hex River Mountains, 1 900 m, (–BA), 26 Oct. 1997 H. P. Linder 6799 (ZH, holo.; BOL, E, G, K, MO, NBG, NSW, PRE).

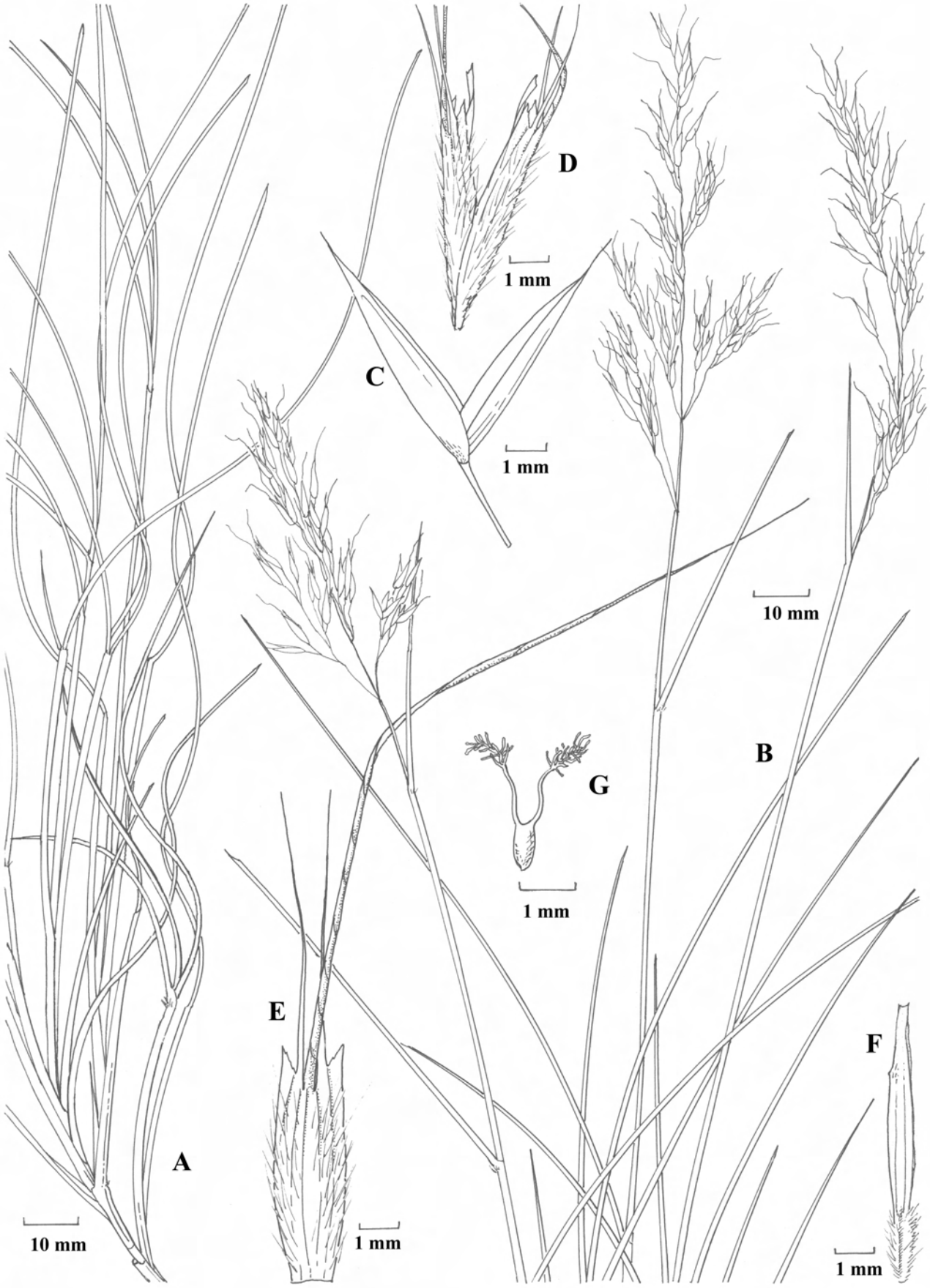
Perennial, caespitose or mat-forming; older plants forming ‘fairy rings’ (with the centre of the ring dying), up to several metres diam.; without multicellular glands. Culms 150---400 mm tall; nodes glabrous; basal sheaths white shiny, persistent; prophylls truncate, often bilobed, upper margin ciliate or bristly, keels remaining parallel to apex, scaberulous or dentate, extended into 20 mm long awns; innovation buds intravaginal. Leaves cauline; sheaths glabrous; sheath mouth glabrous; ligules 0.5---1.0 mm long fringe of hairs; blades 150---200 x1 mm, rolled, rigid, with sparse, villous hairs at the base of the blade; apex pungent, margins smooth; old blades persisting entire. Inflorescences widely paniculate, open at anthesis (soon closing again), with 30---60 spikelets, 70---90 x 25---50 mm; pedicels mostly patent, shorter than spikelets; inflorescence branches as long as or longer than spikelets, scaberulous; nodes sparsely hairy, puberulous to villous. Spikelets 2-flowered, 7.5 mm long. Lower glume longer than florets, acute to acuminate, 1-veined, 6.5---7.5 mm long, pale green; margins scaberulous, same texture as body of glumes; upper glume similar to lower glume. Lemma 2.25---3.0 mm long, with scattered hairs on the back; veins 7, anastomosing near apex; lemma lobes acute, 1.1 mm long, shorter than the lemma body; lemma awn geniculate, 8 mm long, column twisted, 3 mm long thus as long as lemma lobe setae;. Palea glabrous, linear to lorate, apex rounded to acute or bi-lobed, 3.5 x 0.5 mm,

longer than lemma; keels parallel, glabrous. Lodicules without microhairs or bristles, obtriangular, 3- veined. Anthers 2.1---2.8 mm long. Ovary stalked, turbinate; styles two. Figure 3. Flowering time October to November.

Etymology: the species epithet horrida is named after the prickly, pungent leaves.

Diagnostic characteristics: the linear inflorescence, pungent leaves and cushion habit associate this species with *Pentaschistis rigidissima* Pilg. ex H.P.Linder. Specimens of *P. horrida* were previously included within this species and were considered as one extreme of a continuous gradient of size and spinescent variation (Linder & Ellis 1990). There are several differences that separate these species (see Table 3), including their growth form. Although both species may form ‘cushions’ the plant base differs. The new species forms cushions that expand in size over time by means of a branching culm system; this effectively moves the living part of the plant outwards from the cushion centre, which eventually dies, so that a whole plant forms a ‘fairy ring’. *P. rigidissima*, by contrast, always forms small tufts, rather like a shaving brush, and never forms rings. They have been found in sympatry at several localities, and the morphological and habit differences were consistently maintained. Lastly, phylogenetic analysis of chloroplast data places these two species in different clades (Galley & Linder 2007). *P. horrida* is sister to *P. rosea*.

Figure 3. (following page) *Pentaschistis horrida*, Linder 6799: A, plant base and lower plant; B, upper plant and inflorescence; C, glume pair; D, florets; E, lemma showing setae and awn; F, palea with callus; G, ovary and styles. Artist: Claire Linder-Smith.


A
10 mm
B
10 mm
C
1 mm
D
1 mm
E
1 mm
F
1 mm
G
1 mm

Distribution and habitat: *P. horrida* has been collected from the inland ranges of the Cape Fold Belt, from the Cederberg to the Great Swartberg (Figure 2). All populations occur in soils derived from sandstones. These areas are not only dry, but have a more continental climate with severe frost in winter and fierce heat and a long dry period in summer. The plants grow in soil in open vegetation, sometimes on open plains (such as on the summit of the Rooihoogte Pass over the Waboomsberg, and on the summit plateau of Wolfberg in the Cederberg), and sometimes on steep slopes (such as on the Baviaansberg in the Koue Bokkeveld). *P. rigidissima* by contrast is found more towards the coastal areas on the coastal ranges of the Cape Fold Belt, where the climate is more mesic. There are also microhabitat differences. *P. rigidissima* is restricted to crevices in the sandstone rocks and boulders, often in shady, cool and protected places along the sides of boulders or even underneath them. In contrast, *P. horrida* is found in open areas, or at most between boulders, but never in crevices.

Other specimens examined: WESTERN CAPE.—3219 (Wuppertal): central Cederberg, Sleeppad Hut to Sneeukop, 1 500---1 900 m (–AC), H.P. Linder 4464 (BOL); Bokkeveld, Tafelberg 5 500' (1 676 m) (–CD), Esterhuysen 3931 (BOL). 3319 (Worcester): top of Rooihoogte Pass, 1 234m, 33036'12"S, 19051'02" E, (–BD), C.A. Galley 374 (ZH, BOL). 3320 (Montagu): south of Matroosberg station, 3 800' (1 158 m) (–BD), Acocks 19088 (BOL). 3321 (Ladismith): Towerkop, 1 750 m 33027 '42"S, 21013'01" E, (–AC), Linder, Hardy & Moline L7410 (BOL); Toverkop, Swartberg, (–AC), Esterhuysen 26744 (BOL); Sewe Weeks Poort, 2 000 m (–AD), Linder 5486 (BOL); Montagu to Matroosberg, 3 700' (1 128 m), MRL 370 (BOL); Swartberg Pass (–BD), Barker 999 (BOL): Great Swartberg, 1 800 m (–BD), Linder 4571 (BOL). 3322 (Prince Albert): Zwartbergen, 4 000' (1 219 m) (–AC), Bolus 11673 (BOL); 3323 (Uniondale): Kouga Mountains, 5 500' (1 676 m) (–DA), Esterhuysen 27974 (BOL).

Table 3. Comparison between *Pentaschistis horrida* and *P. rigidissima*

	P. horrida	*P. rigidissima*
culm length	270---400 mm	150---200 mm
leaf length	150---300 mm	up to 100 mm
leaf indumentum	sparsely villous at the base of the leaves on the upper surface	glabrous

Pentaschistis juncifolia

Pentaschistis juncifolia was originally described by Stapf in Flora capensis (1899), and is distinct from *P. eriostoma* (Nees) Stapf based on the absence of densely woolly or villous leaf sheaths. Linder & Ellis (1990) sank this species into *P. eriostoma* on the basis of similar spikelets and inflorescences, regarding it as a local form of the very variable *P. eriostoma*. After recent fieldwork, however, we are now convinced that these taxa are indeed distinct, and that *P. juncifolia* should be recognized as a separate species. The most striking difference between the two taxa is the absence of a woolly indumentum at the leaf sheath mouth in *P. juncifolia*. Aside from this, *P. juncifolia* can be recognized as distinct from *P. eriostoma* in the field as it forms much more neatly defined tussocks and has a much paler inflorescence. The habitats of the two taxa differ, with *P. eriostoma* occurring on shales and sandstones and *P. juncifolia* associated with eroded silcrete surfaces on the coastal plains between Bredasdorp and Riversdale. They were observed in sympatry near a silcrete outcrop (Verkykerskop) along the road from Malgas to Heidelberg (34°13'25" S, 20°43'14" E) and were both abundant, maintaining morphological and habitat distinctions. This morphological and ecological evidence is backed up by phylogenetic analysis of chloroplast data where P. eriostoma and *P. juncifolia* are separate on the cladogram and *P. juncifolia* is sister to a large clade that includes *P. eriostoma* (Galley & Linder 2007). *P. juncifolia* has been collected in flower in early October.

WESTERN CAPE.—3420 (Bredasdorp): Swellendam Division, Buffelsjachtsrivier, 1000---2000ft (305---610 m), Zeyher 4545 (K!); road from De Hoop to Malahas, 141 m, 34021'44"S, 20028'45" E; (–AD), 7 Oct. 2003, C.A.Galley 341 (ZH, BOL). 3421 (Riversdale): hills near Zoetmelksrivier, Burchell 6761 (K!); hills near Zoetmelksrivier, Burchell 6750 (K, lectotype!).

ACKNOWLEDGEMENTS

Thanks to Lynn Fish, and Nicola Bergh, Anthony Verboom and the Bolus herbarium for assistance with fieldwork, and to Claire Linder-Smith for the elegant illustrations. Work was funded by the University of Zurich, the Swiss Science Foundation, the Swiss Academy of Sciences and the ‘Georges und Antoine Claraz-Schenkung’.

REFERENCES CITED

Conert, H. J. & Türper, A. M. 1969 *Karroochloa*, eine neue Gattung der Gramineen (Poaceae, Arundinoideae: Danthonieae). *Senckenbergiana Biologica* **50**, 289-318.

Conert, H. J. & Türper, A. M. 1974 Revision der Gattung *Schismus* (Poaceae: Arundiniodeae: Danthonieae). *Abhandlungen der Senckenbergischen Naturforschenden Gesellschaft* **532**, 1-81.

Galley, C. & Linder, H. P. 2007 The phylogeny of the *Pentaschistis* clade (Danthonioideae, Poaceae) based on cpDNA, and the evolution and loss of complex characters. *Evolution* **61**, 864-884.

Goldblatt, P. 1978 An analysis of the flora of Southern Africa: its characteristics, relationships, and origins. *Annals of the Missouri Botanical Garden* **65**, 369-436.

Goldblatt, P. & Manning, J. 2000 *Cape Plants. A conspectus of the Cape flora of South Africa*. Pretoria: National Botanical Institute.

Jürgens, N. 1991 A new approach to the Namib region I: Phytogeographic subdivision. *Vegetacio* **97**, 21-38.

Linder, H. P. 1989 Grasses in the Cape Floristic Region: Phytogeographical implications. *South African Journal of Science* **85**, 502-505.

Linder, H. P. & Davidse, D. 1997 The systematics of *Tribolium* Desv. (Danthonieae: Poaceae). *Botanische Jahrbüche* **119**, 445-507.

Linder, H. P. & Ellis, R. P. 1990 A revision of *Pentaschistis* (Arundineae: Poaceae). *Contributions from the Bolus Herbarium* **12**, 1-124.

Phillips, S. M. 1986 Four new grasses from northeast tropical Africa. *Kew Bulletin* **41**, 1027-1030.

Phillips, S. M. 1994 Variation in the *Pentaschistis pictigluma* complex (Gramineae). In *XIIIth Plenary Meeting AETFAT, Malawi*, vol. 1 (ed. J. H. Seyani & A. C. Chikuni), pp. 359-372. Zomba, Malawi: National Herbarium and Botanic Gardens of Malawi.

Phillips, S. M. 1995 A new species of *Pentaschistis* (Gramineae) from Ethiopia. *Kew Bulletin* **50**, 615-617.

Stapf, O. 1899 Volume VII Gramineae. In *Flora Capensis* (ed. W. T. Thiselton-Dyer), pp. 1-791. London: Lovell Reeve and Co.

Geographical affinities of the Cape flora, South Africa

CHLOE GALLEY AND H. PETER LINDER

Institute of Systematic Botany, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland

Journal of Biogeography 2006, **33:** 236-250

ABSTRACT

The flora characteristic of the Cape Floristic Region (CFR) is dominated by a relatively small number of clades that have been proposed as ‘Cape clades’. These clades have variously been suggested to have African or Austral affinities. Here we evaluate the support for these conflicting hypotheses. We further test the hypothesis that these clades share a common time of differentiation from their geographical neighbours. We use both published and unpublished phylogenetic information to investigate the geographical sister-areas of the Cape clades as well as timing and the direction of biogeographical disjunctions. Almost half of the Cape clades for which unambiguous sister-areas could be established show a trans-Indian Ocean disjunction. The earliest trans-Indian Ocean disjunction dates from 80 Mya. Other disjunctions date from various times in the Cenozoic, and we suggest the process of recruiting lineages into the Cape flora might be on-going. Relatively few Cape clades show a sister-relationship to South America and tropical Africa, despite their relative geographical proximity. Numerous Cape clades contain species also found on the tropical African mountains; in all cases tested, these are shown to be embedded within the Cape clades. While many Cape clades show a relationship to the Eurasian temperate flora, this is complicated by their presence in tropical Africa. The single case study addressing this to date suggests the Cape clade is nested within a European grade. Although many Cape clades show Austral rather than African relationships, there are numerous other patterns suggestive of a cosmopolitan flora. This spatial variation is echoed in the temporal data where, although there is wide variance around the dates of disjunctions, it is clear the Cape flora has been assembled over a long time period. There is no simple hypothesis that can account for the geographical sources of the currently distinctive Cape flora. The phylogenetic positions of Afromontane members of Cape clades suggest a history of dispersal from the CFR, rather than the reverse.

Keywords: Cape flora, Gondwana, disjunct distribution, dispersal, vicariance, biogeography, Africa

INTRODUCTION

The current flora of the southwest tip of Africa is sharply distinct from that of the surrounding areas. Not only are almost 70% of the species endemic to this region (Goldblatt & Manning 2002), but the dominant families and vegetation structure of the region are also very different (Bolus 1886; Adamson 1958; Goldblatt 1978). This led to the early biogeographical recognition of the region (Bolus 1886; Marloth 1908), a spatial and taxic definition that is still largely upheld as the Cape Kingdom (Takhtajan 1986) or the Cape Floristic Region (CFR) (Goldblatt 1978). The CFR has seen much research into the ecology (e.g. Cowling 1992), the remarkable species richness and taxonomic composition (Goldblatt 1978; Cowling *et al.* 1996; Goldblatt & Manning 2002), and speciation (Linder 1985; Cowling 1987; Johnson 1995) of its flora. Recently, the area has also become a model for developing new approaches to biotic conservation designed to deal with climatic change (e.g. Cowling & Pressey 2003).

The flora of the CFR can be divided into three major elements. The first are those clades that are most diverse in the semi-arid Succulent Karoo to the north with outliers in the CFR clades. Typical clades are the *Mesembryanthema* (Aizoaceae p.p.), *Crassula* and several taxa of Asteraceae. The presence of this element is used to argue for a close biogeographical connection between the flora of the CFR and that of the Succulent Karoo, the "Greater Cape flora" of Jürgens (1991; 1997). Secondly, there is the element shared with tropical Africa, and which is most species-rich in subtropical and tropical Africa. Typical clades are *Rhus* (Linder *et al.* 1992), *Aloe*, and many of the genera typical of thicket and forest vegetation in the CFR. Finally, there are the ‘Cape clades’, defined by Linder (2003) as the most inclusive clades with at least 50% of their species in the CFR, and for which the basal nodes are optimised as being located in the CFR. These clades all have similar distributions and patterns of species richness within the CFR (Levyns 1952) and are dominant components of fynbos vegetation. They include among others *Erica*, Proteae, Bruniaceae, Restionaceae p.p., and *Phylica*.

While the first two elements are clearly African, the geographical relationships of the Cape clades are not yet known, and here we investigate these using phylogenetic information. The historical affinities of the Cape clades, whether they are essentially Gondwanan or African, have been debated without resolution (Adamson 1958; Levyns 1964a; Axelrod & Raven 1978; Goldblatt 1978). Linder *et al.* (1992) synthesized these into an African, a Boreal and a Gondwanan track.

The Gondwanan track was first suggested by Hooker (1860) and Bolus (1886), and was later recognised as an "antarctic element" (Levyns 1962;1964b) and incorporated in Croizat's southern track (Croizat 1958). Geographically, the most important links are with Australia and New Zealand, and cited cases usually include Proteaceae, Rutaceae and Restionaceae (Levyns 1952), *Cunonia* (Bradford 2002), *Podocarpus* and *Widdringtonia* (Goldblatt 1978). The nature of the southern relationship, however, remains unsettled. It is argued that taxa such as Proteaceae (Goldblatt 1978), Restionaceae, *Tetraria*, and *Phylica* (Levyns 1964b) have their most primitive African members not in the CFR, but further north in tropical Africa. For these taxa an entry point into Africa closer to the equator is suggested, followed by a southward migration and possibly extinction of some species in the north. Other biogeographers preferred a direct connection between the CFR and Australasia, that is not routed via tropical Africa (Johnson & Briggs 1975; Johnson & Briggs 1981; Linder 1987). However, the process of establishment of this direct southern connection is also not clear.

The African track has been stressed as evidence of a tropical African origin of the Cape flora (Levyns 1938;1952;1964a; Axelrod & Raven 1978), as the number of taxa with tropical affinities is believed to outweigh those with Gondwanan links (Levyns 1964a). Levyns (1964b) suggested that the Cape flora was largely the result of southward migration from tropical Africa to the CFR, whereas Adamson (1958) and Wild (1964; 1968) suggested that the Cape flora was once more widespread in Africa, and differentiated vicariously on the tropical mountains and within the CFR. A southward migration from tropical Africa has even been postulated for taxa with current distributions in the CFR and Australasia (Levyns 1958;1964b).

The boreal track, usually in the form of southward migration from Europe along the higher African mountains to the CFR, is postulated for *Muraltia* (Levyns 1954), *Lobostemon* (Hilger & Böhle 2000), *Cliffortia*, *Erica*, *Stoebe* (Levyns 1964a) and *Moraea* (Adamson 1958), with subsequent extinction in certain cases in the rest of Africa (Levyns 1964).

Goldblatt (1978) assembled lists of genera shared with other regions, and so demonstrated numerous relationships to most parts of the world. Here we use Cape clades to examine and describe the geographic relationships of the current Cape flora with other regions. We focus on extant species and use data from phylogenetic analyses. The use of phylogenies allows more precise and accurate delimitation of the Cape clades and also their sister-clades than would be possible with formal taxonomic ranks (genera, tribes, families). Consequently sister-area relationships are defined more precisely. Furthermore, where sampling and resolution of analyses permit, directionality of relationships between regions

may be inferred and an indication of dates of biogeographic disjunctions may be estimated. Specifically we use phylogenetic information on Cape clades (Linder 2003) and their hypothesised sister-clades, to address the following questions:

- What evidence is there for the hypothesised tracks, and are they sufficient to account for the multitude of biogeographical relationships of the Cape clades?
- Are we able to interpret the origins of the Cape flora from the directionality of any of these relationships?
- Was there a particular time period when most Cape clades diverged from their geographic distinct sister-clades?
- Were elements common to the Cape and Afromontane floras derived from the CFR, or *vice versa*?

METHODS

The Cape flora clades are defined as the most inclusive monophyletic group geographically rooted in the Cape flora. Consequently the sister-clade of each Cape clade has to be centred outside the CFR, and so can provide an indication of the geographical affinities of each Cape clade. We surveyed the phylogenetic literature to establish the sister-clades of all of the Cape clades, as well as *Prionium*. Only proposed clades which appeared to be non-monophyletic, and clades for which no phylogenetic information was available, were omitted. The geographical ranges of the sister-clades were documented from Mabberley (1997), Germizhuizen & Meyer (2003), and relevant floristic or monographic literature. Species taxonomy and counts of species occurring in the CFR follow Goldblatt & Manning (2000) or more recent monographs where available.

Geographical areas were optimised onto the node that subtends the Cape clade to locate the geographical origin of the Cape clades. This was carried out by hand and conclusions were drawn in cases where the outcome of optimisation was unambiguous. Where there is doubt that would alter the conclusions reached, this is mentioned in individual cases. On its own a sister relationship does not inform us about the direction of a biogeographic relationship and the node below the Cape clade and the sister clade must be known. Only in cases where there was sufficient resolution and taxon sampling was it possible to do this and infer direction of the relationship.

In the almost complete absence of fossil material from the CFR (Levyns 1964a; Linder 2003), molecular dating is the only source of information as to when the Cape clades might have differentiated from their extra-CFR sister-clades. In a few cases, we located references in the literature on the age of the node subtending the Cape clade. In most instances, this age was inferred by reference to secondary calibration dates, mostly from Wikström *et al.* (2001). While we are very aware of the large error margins associated with such estimates (Graur & Martin 2004), this work constitutes a first attempt to date the origins of the Cape clades.

RESULTS

Here we report only the results and interpretation of data that could be interpreted unambiguously. Results for clades that resulted in ambiguous interpretations are presented in the appendix.

We were able to establish the sister clades of 25 out of 33 Cape clades identified by Linder (2003) plus *Prionium* (see Appendix). These suggest that the current Cape flora has biogeographic affinities with Australasia, South America and tropical Africa. No clade shows an unambiguous direct relationship to the northern temperate regions. For many clades the data are not adequate to determine unambiguous relations. They are shown as 'shared' between areas in Fig. 1. Furthermore due to insufficiently resolved phylogenies or inadequate sampling, the sister clades of Gnideae, Asteraceae p.p., *Centella* and *Muraltia* could not be determined.

Area relationships

Trans-Indian Ocean relationships

The trans-Indian Ocean relationship of the CFR is evident from four clades. The sister clades of *Ehrharta*, the *Pentaschistis* clade, the African Restionaceae and Geissolomataceae occur in Australasia (Fig. 2). The *Pentaschistis* clade is sister to the rest of the Danthonioideae minus a *Merxmuellera* grade (Barker *et al.* 2000) and all tropical African taxa tested so far are well nested within the Cape group (unpubl. data). This supports a relationship of the CFR to the Australasian south temperate regions, although the *Merxmuellera* grade is of the tropical African group. The Cape *Ehrharta* species form a clade nested in the rest of the tribe, which is widely distributed in Australia, New Zealand, Malesia and the southeast

Pacific (Verboom *et al.* 2003). A sister-group relationship between the African and Australian Restionaceae has been demonstrated (Johnson & Briggs 1981; Linder *et al.* 2000; Linder *et al.* 2003) and likewise *Geissoloma* is sister to a clade of *Ixerba* from New Zealand and *Strasburgeria* from New Caledonia (Sosa & Chase 2003).

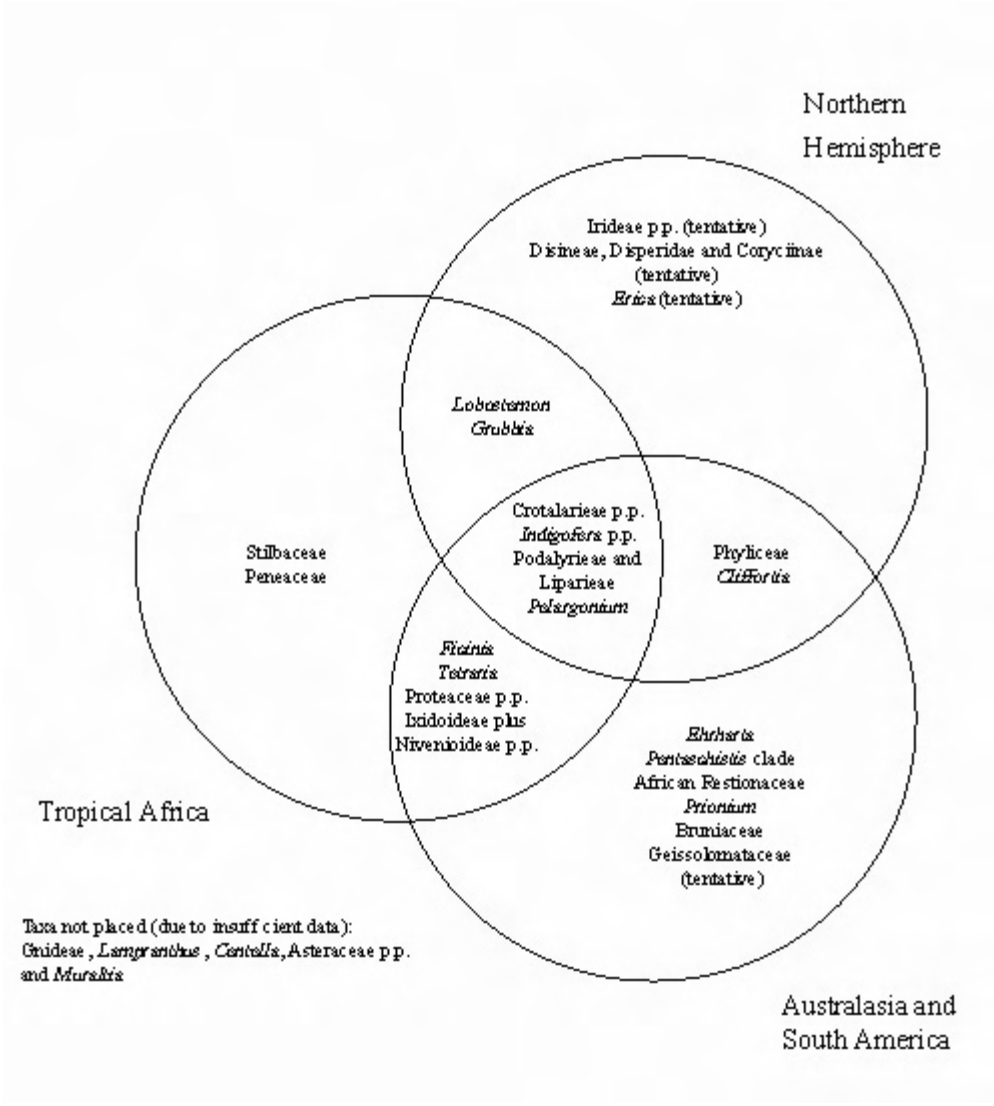
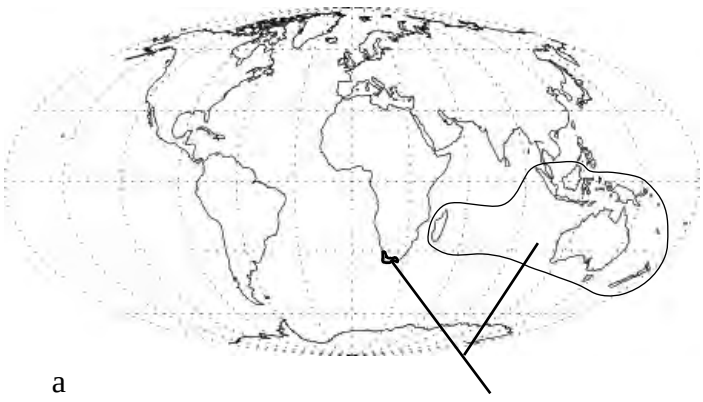
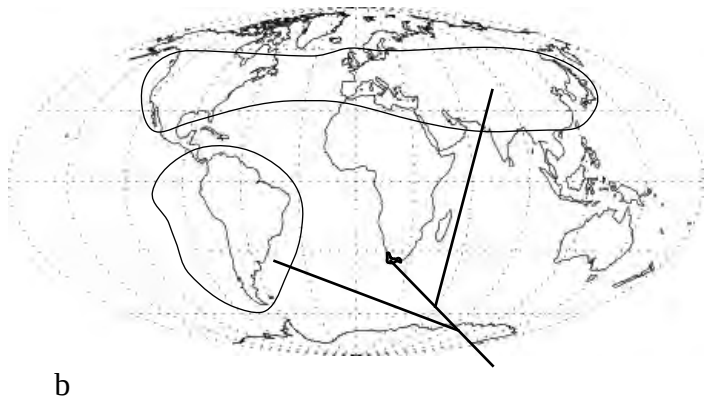


Figure 1. Venn-diagram summarising the area relationships manifested by sister-clades to Cape clades for the relationships examined herein. Where the available data are insufficient to reject alternative area relationships, the clades are placed in the intersection of these areas. Groups for which sister-relationships were not established (*Centella*, *Muraltia*, Asteraceae p.p., Gnideae p.p., *Lampranthus*) are not included.

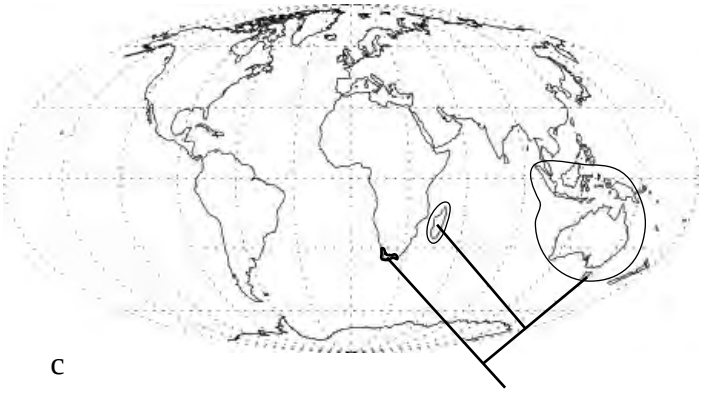
Figure 2. (following page) Simplified area relationships for each Cape clade; Mollweide projection. a. *Tetraria*; b. Irideae p.p.; c. Crocoideae and Nivenioideae p.p.; d. *Ehrharta*; e. Bruniaceae; f. *Geissoloma*; g. Stilbaceae, h. African Restionaceae, illustrating that this clade originated in Australasia. For the Cape clades only the CFR is indicated (although the distribution of members within may extend further, this is not indicated), for the sister-clades the full distribution range is outlined.



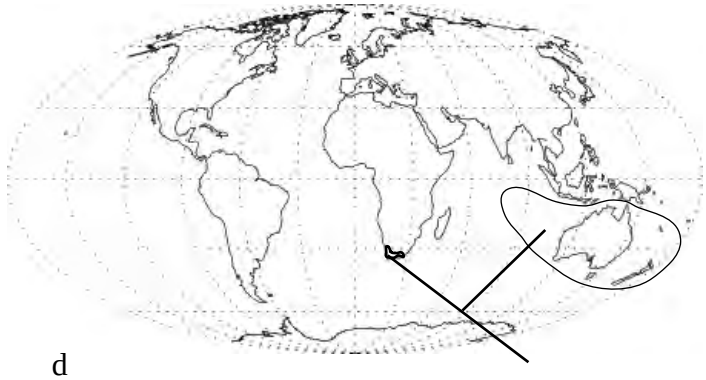
a



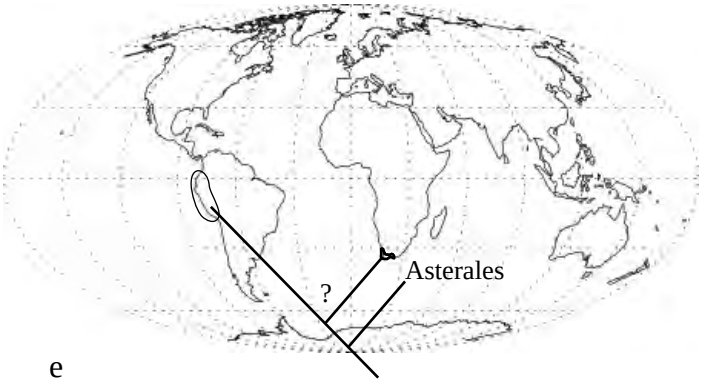
b



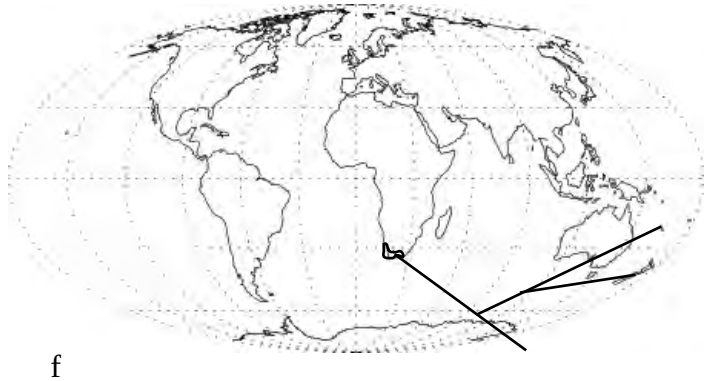
c



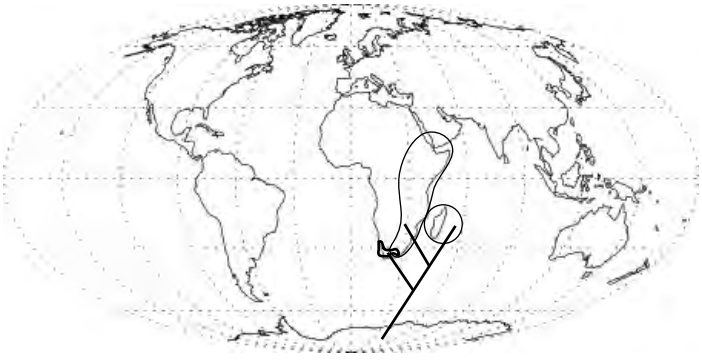
d


Asterales
?

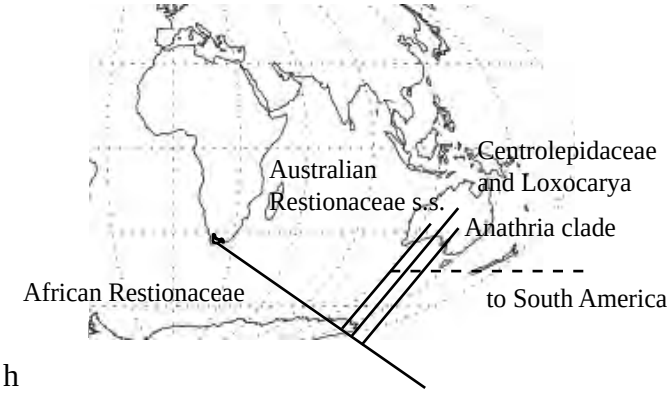
e



f



g


Australian
Restionaceae s.s.
Centrolepidaceae
and Loxocarya
Anathria clade
to South America
African Restionaceae

h

A trans-Indian Ocean disjunction may be demonstrated by several clades in the Proteaceae. Firstly, *Brabejum* is more closely related to the Australian Macadamieae than to the Proteae. Secondly, the African Proteae form a polyphyletic group, with each clade related to Australian taxa, thus showing several Afro / Australian links (Hoot & Douglas 1998). However, without further data it is not possible to rule out the possibility that tropical African Proteae are sister to the Cape clade (rather than embedded within it). This is also the case for *Tetraria* (Appendix and Figs. 1 and 2). In these cases insufficiently resolved phylogenetic hypotheses mean that alternative geographical connections cannot be rejected. Phyliceae are also problematic, since they are related to both North American and Australian taxa.

Trans-South Atlantic Ocean relationships

Among the clades considered, the relationship between the CFR and South America is represented only by *Prionium*, which is sister to the South American *Thurnia* (Givnish *et al.* 1999). Although *Prionium* is not endemic to the CFR, its outlier populations occur in association with other Cape elements, and as such it is here regarded as a Cape element. Bruniaceae possibly represents trans South-Atlantic disjunction but the phylogenetic position of the family remains enigmatic. Molecular analyses suggest a sister relationship to the Northern Andean family Columelliaceae (Bremer *et al.* 2002) (Fig. 2), but the possibility remains that it could also be related to some other unplaced small Southern Hemisphere families, such as Sphenostemonaceae, Tribleaceae, Eremosynaceae and Escalloniaceae (Marcus Quint, personal communication).

Tropical African relationships

An unambiguous sister-group relationship between tropical Africa and the CFR is demonstrated by two Cape clades: Penaeaceae and Stilbaceae. Cladistic analyses of molecular data suggest the sister to Penaeaceae is the Southern and tropical African *Olinia* (Oliniaceae). The sister to these two families is *Rhynchocalyx*, an eastern South African genus (Schönenberger & Conti 2003). Stilbaceae (including Retziaceae), is sister to the widespread African *Nuxia* (Backlund *et al.* 2000) (Fig. 2).

There are still Cape clades that could strengthen either of the patterns observed above but which have not been adequately investigated. These include the *Ficina*, *Tetraria* and Proteae clades, plus Irideae p.p. (Fig. 2), Crocoideae and Nivenioideae (sensu Goldblatt 1990) p.p. (Fig. 2), and *Lobostemon*. In all these cases more thorough sampling of the tropical African species is necessary to confirm that they are nested within the Cape groups.

Eurasian relationships

Several Cape clades indicate a relationship between the CFR and Eurasia: *Erica*, Satyriinae, Disineae, Grubbiaceae, Irideae p.p., *Cliffortia*, *Lobostemon*, and *Pelargonium*. The only study that has dealt specifically with this problem concerns *Erica*. Although sampling was low, the Cape members are shown to be nested within a European grade. It is therefore concluded that the Cape clade is derived from a European group rather than *vice versa* (McGuire & Kron 2005). Only *E. arborea* was sampled to represent tropical species thus little can be said about the process of colonisation of Africa.

Regarding other clades the strength of evidence is compromised by either weak support of phylogenetic relationships or inadequate sampling of tropical African members of these clades.

Timing

The separation of Cape lineages from their sister-clades (presumably dating the vicariance or dispersal events) is spread from the Late Cretaceous (max. 92 ± 6 mya) to the present, without any distinct patterning (Table 1). Although the variance in the divergence ages is enormous, it is clear that splits between Cape lineages and sister taxa did not occur within a narrow time frame.

Table 1 (following page) Age estimates for the split between the CFR and sister lineages for each Cape clade. Dates obtained directly from Wikström *et al.* (2001) (abbreviated as "W") are given as the estimate from maximum likelihood ± SE. NPRS = non parametric rate smoothing (Sanderson 1997), MRCA = most recent common ancestor.

Cape clade	estimated age of divergence of Cape lineage (mya)	methodological notes
Grubbiaceae	92 ± 6 to 57 ± 8	Sampling in W prohibits direct estimation of the age of the node shared by Grubbiaceae and the rest of the Cornaceae. Instead we use relative node positions on chronograms from Fan and Xiang (2003) to infer an age bracket containing that contains the node representing the split between the Northern Hemisphere Cornaceae and Grubbiaceae. Temporally this node lies between the most recent common ancestor of the nyssoids and *Cornus* - *Alangium*, and the most recent common ancestor of *Nyssa* and *Camptotheca* (Fig. 6), nodes that are sampled in W.
Geissolom-ataceae	81 ± 5	The node between Aphloiaceae and Ixerbaceae as sampled in W is inclusive of the MRCA between Geissolomataceae and Ixerbaceae / Strasburgeriaceae, offering a maximum age for the estimation of the Cape disjunction.
African Restionaceae	60-70	*Milfordia* pollen with affinity to the African Restionaceae has been identified in the Arnot Pipe, Northern Cape (Scholtz 1985), offering a minimum age for the occurrence of Restionaceae in South Africa.
Proteeae p.p.	60-70	Although the composition of the Cape clade(s) within this family are not known, there are two palynological records of Proteaceae in the Paleocene Arnot Pipe fossil deposit (Scholtz 1985), *Triporopollenites namaquensis* and *Propylipollis meyeri*. Although the composition of the Cape clade of Proteeae is unknown we have a minimum age for the occurrence of Proteeae in Southern Africa.
Stilbaceae	49 ± 3	The node between Stilbaceae and the sister group of Lamiaceae plus Schrophulariaceae p.p. provides a maximum age for the *Nuxia* / Stilbaceae divergence (W).
Nivenioideae and Ixioideae	46	Goldblatt *et al.* (2002) used W to calibrate a chronogram of Iridaceae. We use the node between the Cape clade and the rest of the family.
Irideae p.p.	42	Goldblatt *et al.* (2002) used W to calibrate a chronogram of Iridaceae. We consider the node representing the split between the Cape clade and the Northern Hemisphere *Iris*, *Belamcanda* and *Pardanthopsis*.
Pelargonium	41 ± 3	W date the split of *Pelargonium* from the rest of Geraniaceae to 41 ±3 million years. We assume the whole genus to be a Cape clade thus we apply this date to the Cape / Northern Hemisphere or Cape / Southern Africa split.
Ehrharta	37	Verboom *et al.* (2003) used the suggested age of Ehrhartoideae of 41 million years, derived from a secondary date for the Ehrhartoid – Poaceae divergence from Bremer (2002) to estimate a calibrated divergence rate. We used this to age the African / Australian lineage split.
Prionium	20-56 (95%CI)	Five fossil calibration points within the Poales are used to date the node between *Thurnia* and *Prionium* (Bremer 2002).
Crotalarieae p.p. and Podalyrieae plus Liparieae	23.7 - 32.6	The "earliest unequivocal fossil evidence for Leguminosae" (Wojciechowski 2003) has been used to age the origin of the core Genisoids (differences shown are from divergence different analyses used). Both these Cape clades are members of this group thus this offers a maximum estimate for the ages of these groups.
Indigofera p.p.	20.91 ± 3.02 to 26.28 ± 2.47 (±SE)	Schrire *et al.* (2003) used a secondary calibration point from Wojciechowski (2003) to calibrate a chronogram of *Indigodera*. The node representing the split between the Cape clade and the pantropical clade is not aged but is temporally between the nodes that represent the radiation of *Indigofera* and the boreotropical crown group. This gives an age bracket of 20.91 to 26.28 mya (using NPRS) for the minimum age of the split of the Cape clade from the sister group.
Phyliceae	15-18 to 26	Richardson *et al.* (2001b) used the assumed dispersal of *Phylica* species to Réunion and St. Helena (2 and 14.3 mya respectively) to calibrate the chronogram of this group, thus yielding maximum ages. Dating is only considered within the Cape clade with the diversification age of the Phyliceae estimated as 15-18 mya. W estimate the youngest node within Rhamnaceae that is still inclusive of the Cape group as 26 million years old. This provides an upper limit to the stem of the Phyliceae.

Upland tropical Africa

Many Cape clades also have species in upland tropical Africa, where they form part of the Afromontane flora (Weimarck 1933;1936). Where these were included in phylogenetic analyses we were able to test whether these are nested within the Cape clades, or are basal members to the Cape clades. Data for *Cliffortia* (Whitehouse 2002) (Fig. 3), Phyliceae, Irideae p.p. (Goldblatt *et al.* 2002), *Aristea* (Goldblatt & Le Thomas 1997; Goldblatt *et al.* 2004), Diseae (Linder 1994), *Pentaschistis* (unpubl. data), *Pelargonium* (Bakker *et al.* 1998) and the African Restionaceae (Hardy and Linder pers. comm.) clearly demonstrate that the tropical species analysed so far are nested within the Cape clades.

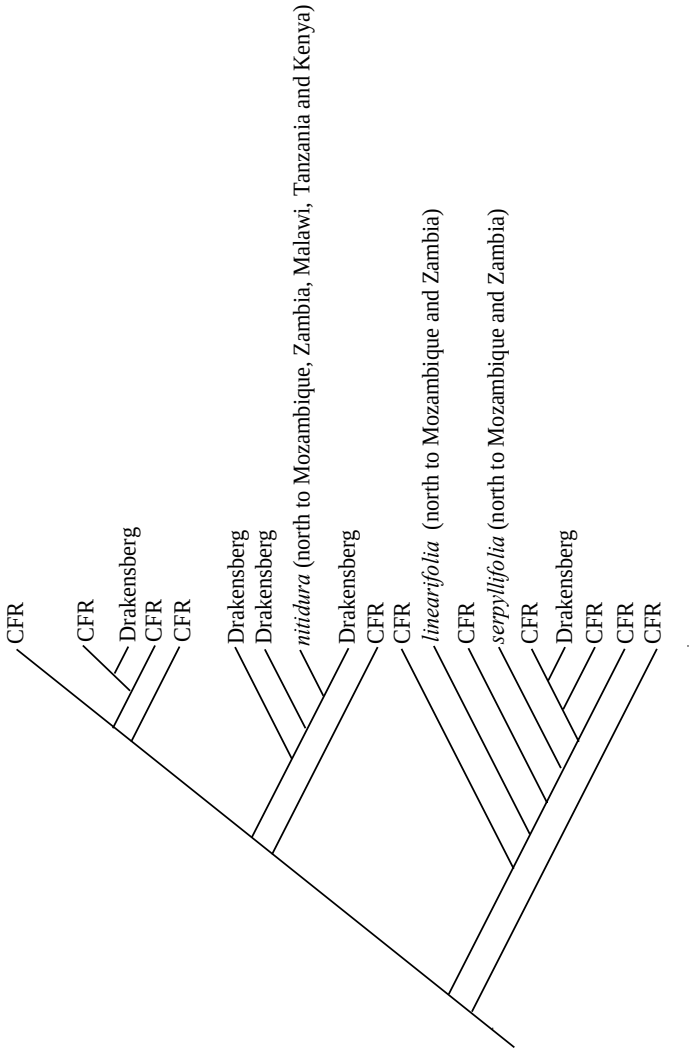


Figure 3. Area-cladogram for *Cliffortia* illustrating the nesting of tropical African taxa within the Cape group. CFR = Cape Floristic Region, Drakensberg = region and eastern Southern Africa. Taxa in tropical east Africa are specified (modified from Whitehouse 2002).

DISCUSSION

Quality of the data

Phylogenetic hypotheses were available for clades including just over 35% of the total number of Angiosperm species in the Cape Floristic Region. However, as in some cases the distribution of sister-clades is widespread, not all of these clades are biogeographically informative. The phylogenetic spread of the remaining clades that are informative for our problem is rather imbalanced, with ~58% of monocotyledons but less than 30% of dicotyledons accounted for. In recent years there has been an explosion in the quantity of phylogenetic data, especially those based on molecular studies, but conspicuous gaps in the data still remain, including Asteraceae, Brassicaceae and Rutaceae, families which make up a significant proportion of the Cape Flora.

Even where phylogenetic hypotheses are available, the critical nodes linking the Cape clades to their sister-clades are often poorly supported. This applies to the *Ficinia* clade, *Centella*, *Grubbia*, *Muraltia* and *Cliffortia*, and indicates that we cannot be confident in some conclusions reached from these cladograms. Secondly, incomplete sampling often means that hypotheses of relationships between different areas cannot be rejected as we cannot rule out all possibilities of species relationships and, therefore, area relationships. For example, in several cases (e.g. *Tetraria*, *Ficinia* and *Lobostemon*) not all tropical African species have been sampled. The possibility therefore remains that these unsampled species may be sister to the main Cape clade rather than nested within it, which would indicate a sister relationship between the CFR and tropical Africa (Fig. 1).

Estimation of node ages contains many sources of error, including errors in branch length estimation, inaccurate topology, incomplete sampling (Linder *et al.* 2005), chronogram calibration (Sanderson 1997), and the compounded error resulting from using secondary calibration points (Graur & Martin 2004). In many cases these error values are not published and thus we are unable to report them. In spite of these issues we demonstrate a very wide range of age estimates for the stem ages of Cape clades, and consequently the error is most likely small in comparison to differences in the date estimates.

Biogeographical relationships of the Cape clades

The presence of **a trans-Indian Ocean element** in the Cape flora, as postulated by Levyns (1964a), Goldblatt (1978) and Linder *et al.* (1992), is corroborated, but we show that it

involves a larger proportion of the Cape flora than previously suggested. Furthermore, there is no evidence that any southern connection was indirect over tropical Africa, as suggested by Levyns (1964a). The tropical African species of all groups for which adequately resolved phylogenetic hypotheses are available are nested within otherwise Cape groups, thus rejecting the hypothesis of Levyns (1964b) that Cape members of these groups are derived from the tropical African species.

Aside from *Prionium* and possibly Bruniaceae there are other examples **of trans-South Atlantic** relationships in the Cape flora, these include *Drosera* (Rivadavia *et al.* 2003) and Haemodoraceae (Hopper *et al.* 1999). However, these are few compared to the trans-Indian Ocean disjunctions. The small number of trans South Atlantic disjunctions is surprising, especially given that South America is closer than Australia (6,000 km vs 8,000 km) to the CFR. This is further accentuated by the relatively large number of disjunctions between tropical Africa and South America (Thorne 1973).

Understanding the possible biogeographical affinity with **European** groups is compounded by the complex tripartite relationship between Eurasia, tropical Africa and the CFR. To date the only phylogenetic hypotheses available that deal directly with this relationship are *Androcymbium* where analysis of cpDNA and karyological data suggest northward dispersal of the group into the Mediterranean from South Africa (Caujapé-Castells, *et al*. 2001), and *Erica*, for which the analyses suggest southward movement from Europe (McGuire & Kron 2005) although the relationships with Tropical African species are largely unknown. Further interesting taxa to analyse here would be *Lotononis* (van Wyk 1991), *Argyrolobium*, *Romulea*, and *Anemone* (*Knowltonia*). It remains unclear in most instances whether the tropical African distributions were established after the Cape – Eurasian disjunction, or whether they are "stepping stones" along a migration route between the two hemispheres.

Origins of the Cape flora

Sister-area relationships (discussed above) do not indicate the directionality of these relationships. In several cases the closest relative of the Cape clade and its sister-clade is a large cosmopolitan group, thus geographic information required to optimise their common node will remain elusive. For only four Cape clades and their sisters are the data adequate to indicate the area of origin. These are all Southern Hemisphere patterns. Two of these indicate

a migration from Australia (*Ehrharta* and the African Restionaceae), one from Africa (Danthonioideae) and one Southern Hemisphere including Madagascar (Crocoideae and Nivenioideae).

Timing

The ages of **trans-Indian Ocean disjunctions** show a very wide range. Proteaceae and Restionaceae were present about 200 km to the north of the present day CFR by 60-70 mya (Scholtz 1985) and the age estimate of the Geissolomataceae suggests an even earlier presence of at least around 81 mya. Conversely much more recent age estimates are inferred for *Ehrharta*, and the Crocoideae / Nivenioideae clade. The southern Indian Ocean opened between 120 and 100 mya (Scotese 1997), consequently Restionaceae, Proteae and Geissolomataceae may be the result of vicariance on dispersal across a proto-Indian Ocean. The Kerguelen Plateau may have also provided a mid ocean stepping stone (Sampson *et al.* 1998; Cooper *et al.* 2001; McLoughlin 2001). Fossil wood and palynological evidence suggest that a rich flora was present on the plateau in the Early to mid Cretaceous, especially Albian and Aptian stages (Francis & Coffin 1992; Mohr & Gee 1992; Schlich & Wise 1992). The palynoflora of this time is similar to Early – mid Cretaceous floras known from southern Gondwana, especially Australia and Antarctica (Mohr & Gee 1992). Situated in the southern Indian Ocean it is suggested that the plateau could have acted as a large stepping stone between between India and Australia – Antarctica (McLoughlin 2001), effectively reducing the distance between Southern Africa and Australia. However, more recent lineage splits require long distance dispersal to explain disjunctions.

The molecular age estimate of the **trans-South Atlantic Ocean** disjunction shown by Thurniaceae suggests a Miocene event, although the Eocene is at the earliest extreme of the dating range. The South Atlantic is a relatively old ocean and the basin started opening ca. 120 mya (Scotese 1997), although the Walvis Ridge may have provided stepping stones until the Eocene (Parrish 1993). Vicariance is therefore a possible albeit unlikely explanation for the Thurniaceae disjunction.

Representing a **tropical African** link, the disjunction with Stilbaceae could be dated to the Eocene. This implies that the differentiation of the Cape flora from that of tropical Africa commenced early, at the very start of the late-Tertiary global cooling and aridification trend (Zachos *et al.* 2001).

There are disparate ages for the **Southern African - European** disjunction. The split between *Pelargonium* and the rest of the Geraniaceae (presumably representative of the northern temperate regions) is dated at ca. 41 mya, while the separation between Grubbiaceae and Cornaceae dates to 91 mya.

There are abundant indications that prominent members of the current Cape flora are old, dating at least to the Eocene, and half of the Cape clades show disjunction ages from their sisters that precede the Oligocene. This also applies to the only dated clade with a tropical African sister-clade. These "old" Cape clades are currently among the most species-rich in the flora: the two Iridaceae clades, Restionaceae, Proteae. While this does not imply that the typical "fynbos" vegetation was in place, nor that these clades dominated the mid-Tertiary flora of the region, it does not preclude this possibility. Conversely, the data show that there are also some very recent disjunctions, which can only be explained as dispersal events. The formation of biogeographic disjunctions still seems to be continuing, and *Pelargonium, Wurmbea* and *Pentaschistis* have Australian members recently derived from Cape groups (Bakker *et al.* 1998; Vinnersten & Reeves 2003). *Pentaschistis insularis*, another recently derived species, sister to the African *Pentaschistis airoides* ssp. *jugorum* is present on Amsterdam Island (unpubl. data), an island half way between Cape Town and Australia. Furthermore, there are a number of other, as yet undated, trans-Indian Ocean disjunctions, e.g. *Bulbinella*, *Caesia*, *Dietes, Villarsia, Metrosideros, Anacampseros, Acaena*, *Cunonia* (Goldblatt 1978). Long distance dispersal is not ubiquitous: there are numerous species of Aizoaceae, Iridaceae, Orchidaceae, Fabaceae, Poaceae and Proteaceae that have been successfully introduced across the Indian Ocean (Scott & Panetta 1993), albeit mostly into disturbed habitats. This indicates that if they had made the crossing without human assistance their establishment may have been successful.

Upland tropical Africa

A clear pattern regarding the origin of the Afromontane flora has been identified. Levyns (1964b) interpreted Cape clade species found here as basal to the Cape radiations, implying a southward migration in the flora. This hypothesis was also supported by Axelrod and Raven (1978) on palaeoclimatic arguments. Adamson (1958) and Wild (1964; 1968) preferred a vicariance explanation, that the modern disjunctions in the African temperate flora are relictual and that the temperate flora once had a more continuous distribution in Africa. Both

of these hypotheses are rejected: for eight Cape clades that have species in the uplands of tropical East Africa the tropical African species sampled are embedded within otherwise Cape lineages. This supports a third explanation, that the Afro-montane taxa with southern connections are derived, in part, from the Cape clades, either from one or several migrations northwards from the CFR (Linder 1994).

Comparisons with other floras

The Cape flora is assembled from clades shared with Australasia, Europe and South America. Cosmopolitanism among floras seems to be the norm, rather than the exception. A broad range of relationships was also documented for the much smaller Afroalpine flora by Hedberg (1965). He demonstrated the presence of Mediterranean, South temperate, North temperate, CFR, as well as more widespread (e.g. pantropical) elements. A similar pattern is found in the Australian flora, also characterised by high species-level endemism. Here Crisp *et al.* (1999) used track analyses to identify South Pacific, equatorial, trans-Indian Ocean and pantemperate elements, leading them to conclude that the Australian flora "contains a representative sampleof the world's flora", a conclusion that is reflected in a compilation of phylogenetic data of Australian vascular plant families (Crisp *et al.* 2004). Likewise the Hawaiian flora, again characterised by high species-level endemism, has been shown to have cosmopolitan affinities (Wagner *et al.* 1990). New Zealand, by contrast, provides an example of a region where much of the flora was largely recruited from Australia, and possibly much of it quite recently by dispersal across the Tasman Sea (McGlone *et al.* 2001; Winkworth *et al.* 2002).

CONCLUSION

There is currently a sharp distinction between the climate and flora of the CFR and that of tropical Africa, but this may not always have been the case. We are here concerned with the affinities of Cape clades as representative of the current Cape flora. The spatial and temporal spread of the groups related to the Cape clades suggest that the Cape flora is assembled from lineages currently found on most continents of the world and that this assembly occurred during the whole of the Tertiary. This precludes simplistic explanations for the Cape flora as an African, or Gondwanan, or Australasian flora. While each clade has a unique history, we suggest this may not the case for floras.

ACKNOWLEDGEMENTS

We thank Chris Hardy, Felix Forest and Marcus Quint for sharing unpublished data. This research was funded by the University of Zurich and the Swiss Science Foundation. We also thank Peter Goldblatt and an anonymous reviewer for comments on the paper.

APPENDIX 1

Data for Cape clades

***Ficinia* plus *Desmoschoenus* (Cyperaceae)**. This clade includes recently transferred *Ficinia trollii* and *F. nodosa* (previously *Isolepis*) (Muasya *et al.* 2000; Muasya *et al.* 2001). Relationships within the clade are not fully resolved. *Isolepis marginata* (Namaqualand to CFR to Eastern Cape and Australia) is sister to the Cape clade and the sister to this group is the rest of *Isolepis* (Muasya *et al.* 2001). *Isolepis* is distributed in Australia, the CFR and tropical Africa (Haines & Lye 1983; Hnatiuk 1990; Goldblatt & Manning 2000). However incomplete sampling of *Isolepis* prohibits optimisation of the node below the Cape clade, and consequently both Australasia and tropical Africa are candidate areas for a sister relationship to the CFR.

***Tetraria* (Cyperaceae)** The genus is centred in the Cape, with few species occurring elsewhere (East and Central Africa two species (Haines & Lye 1983), eastern South Africa ca. eight species (Gordon-Gray 1995; Goldblatt & Manning 2000) and Australia four species (Hnatiuk 1990). We assumed the monophyly of *Tetraria*, and relationships within the genus have not been investigated and therefore we cannot rule out that the tropical African members are sister to the Cape clade, thus that the CFR is sister to a tropical African group. The sister group to *Tetraria* is a clade of *Baumea* and *Lepidosperma*, but this grouping has little support (Muasya *et al.* 2000). These two genera are widespread from Madagascar to the Pacific islands.

Irideae p.p. (Iridaceae) This clade comprises *Moraea* s.l., *Ferraria*, *Dietes* and *Bobartia* and has radiated especially in the more extreme winter-rainfall areas of the Western Cape and also to a lesser extent in the summer rainfall areas in eastern Southern Africa as well as north to Ethiopia. Cladistic analyses show that the tropical African species of *Moraea* are embedded within a Cape clade (Goldblatt *et al.* 2002), but the sampling of non-Cape species is only ~30%. The sister of the African Irideae p.p. clade are the European *Iris*, *Belamcanda* and *Pardanthopsis* (Reeves *et al.* 2001). However, the situation for the African Irideae is unclear since two of the lineages within the clade occur throughout Southern and eastern Africa (*Dietes*) and widespread in tropical Africa (*Ferraria*) as well as in the Cape. This results in area relationships as follows: (*Iris*, *Belamcanda* and *Pardanthopsis,* Europe (*Bobartia*, Cape; *Dietes*, E. and S. Africa and Lord Howe Island) (*Moraea*, Cape; (*Ferraria p.p*., Southern African; *Ferraria glutinosa*, tropical Africa))).

Crocoideae and Nivenioideae (sensu Goldblatt 1990) p.p. (Iridaceae) The Cape clade is well supported by a cladistic analysis of molecular sequence data (Reeves *et al.* 2001). The first member to split off in this clade is *Aristea*. Thirty-three of the 50 species in this genus are from the CFR, the remainder are found throughout sub-Saharan Africa and Madagascar. Cladistic analyses of *Aristea* (Goldblatt & Le Thomas 1997; Goldblatt *et al.* 2004) show that non-CFR species (the subgenus Eucapsulares) form a clade nested within a paraphyletic Cape group. This supports the Crocoideae and Nivenioideae, inclusive of *Aristea*, as a Cape clade. Relationships with closely related taxa are: (((Crocoideae, Nivenioideae p.p.) *Geosiris*) *Patersonia*) (Reeves *et al.* 2001). The distributions of the latter two genera (*Geosiris*: Madagascar, *Patersonia*: Australasian) support a Southern Hemisphere relationship of the CFR.

Disineae, Disperidae and Coryciinae (Orchidaceae) These orchid clades are nested within a larger Diseae - Orchideae (Douzery *et al.* 1999), which is centred in Africa and Europe. The Disineae, Coryciinae s.s. and possibly the Satyriinae are centred in the CFR, and could be regarded as Cape clades, and all three are clearly part of an Afro-European orchid flora.

***Lampranthus* (Aizoaceae)** The phylogenetic hypotheses of the Ruschioideae are still not adequately resolved for biogeographical inferences. Furthermore, *Lampranthus* may not be monophyletic (Klak *et al.* 2003). Consequently it is not clear whether there is a distinctive Cape clade, or whether the Cape members of the group are embedded within the Succulent Karoo centred clade.

***Centella* (Apiaceae)** In addition to one widespread species (*Centella asiatica*) there are four other species found throughout Southern Africa (Germishuizen & Meyer 2003) while the remainder are Cape species. Insufficient sampling in phylogenetic hypotheses means the most closely related genus to *Centella* is unknown (Plunkett *et al.* 1997), thus area optimisation of the node below the Cape clade is not currently possible.

Asteraceae There are several proposed Cape clades of Asteraceae (Levyns 1964a; Linder 2003). The only group to have been investigated phylogenetically is Arctoteae. Most species in this tribe are endemic to the Greater Cape Floristic Region. A recent phylogenetic analysis proposes a framework for the close relatives of this tribe but ambiguity in optimisation and very low sampling means that conclusions cannot be drawn (Funk *et al*. 2004). The Asteraceae are not explored any further here.

***Lobostemon* (including *Echiostachys*) (Boraginaceae)** Molecular (Hilger & Böhle 2000) and palynological (Retief & Van Wyk 1998) evidence suggest that *Echium* is sister

Lobostemon and that *Pontechium* is sister to this pair of genera. Although this clade is centred in the CFR, seven species of the clade also occur in the rest of South Africa, and incomplete sampling in the phylogenic analysis means that we cannot rule out the possibility that widespread South African species are sister to the Cape clade *versus* embedded within it. The distribution of the relatives suggests an Afro-European relationship.

***Erica* (Ericaceae)** The vast majority of species of *Erica s.l.* are endemic to the CFR, but there are also species throughout Africa and Europe. The genus is sister to *Calluna* (Oliver 1991; Kron *et al.* 2002), which is restricted to Europe. Recent phylogenetic analyses show the Cape clade to be nested within a European grade, although the relationship with tropical species is not known (McGuire & Kron 2005). This is consistent with the hypothesis that the Cape *Erica* species are derived from Europe.

Crotalarieae p.p. (Fabaceae) This clade, excluding *Crotalaria*, but including *Lotononis, Lebeckia, Aspalathus, Rafnia, Pearsonia* (Crisp *et al.* 2000), is centred in the CFR, although for some genera most species are found outside the Cape. *Crotalaria* is sister to the clade. Although this genus has especially high species richness in tropical Africa this genus is widespread in the tropics and subtropics, thus offering little biogeographical information without further phylogenetic hypotheses.

***Indigofera* p.p. (Fabaceae)** A portion of this large pantropical genus is centred in the CFR (the "Cape clade"). This clade is sister to most of the rest of the genus (Schrire *et al.* 2003). Due to the widespread distribution of the non-Cape *Indigofera*, no biogeographical interpretation is possible.

Podalyrieae and Liparieae (Fabaceae) This poorly supported (Crisp *et al.* 2000) Cape clade is sister to the tropical African *Calpurnia*, but in the analysis of Van der Bank *et al*. (2002) *Calpurnia* is embedded within this clade and the sisterclade is the widespread genus *Sophora*. Consequently we cannot offer any biogeographical interpretation of this group.

***Pelargonium* (Geraniaceae)** Van der Walt & Vorster (1983) suggested that the Cape *Pelargonium* are derived from tropical African *Pelargonium* because the “most primitive” sections of the genus contain tropical elements. Phylogenetic analyses (Bakker *et al.* 1998) contradict this hypothesis; species from Australasia, Namibia, South Atlantic and eastern Southern Africa sampled so far are well embedded in the remainder of the (Cape) group (Bakker *et al.* 1998). The genus is sister to the rest of the Geraniaceae (Price & Palmer 1993), which is distributed in the Northern Hemisphere (*Erodium* and *Geranium*), Madagascar, S.W. Asia and Southern Africa (*Monsonia* and *Sarcocaulon*). This clade is therefore biogeographically uninformative.

***Grubbia* (Grubbiaceae)** *Grubbia* is sister to *Curtisia* (Fan & Xiang 2003). While Grubbiaceae is endemic to the CFR, *Curtisia* has a wider distribution in Southern Africa. The precise placement within the rest of the Cornales (a generally Northern Hemisphere group) is not robustly resolved, thus precise geographical relationships of the Cape clade cannot be resolved.

***Muraltia* (including *Nylandtia*) (Polygalaceae)** This clade is nested within *Polygala* (Persson 2001), a subcosmopolitan genus. Low species sampling of *Polygala* in phylogenetic analyses render optimisation of the area most closely related to the CFR impossible.

Proteae (Proteaceae) Phylogenetic analysis including three Proteae genera supports polyphyly of this group (Hoot & Douglas 1998). The three Cape genera sampled are closely related to Australian taxa, preliminarily supporting a CFR / Australia relationship. However, there are Cape genera that are distributed through Africa (inc. *Faurea* and *Protea*). Without a species-level phylogenetic hypothesis of the respective genera, it is not possible to reject the hypothesis that the Cape members may be derived from a widespread African group, as suggested by Levyns (1964a). Furthermore, we are currently unable to delimit the Cape clade. Members of the Proteae, however, do illustrate an Afro-Australian relationship.

Phyliceae (Rhamnaceae) Non-Cape species of the Phyliceae sampled (southern Atlantic Ocean and southern Indian Ocean) are well embedded within the Cape clade (Richardson *et al.* 2001a). Phyliceae is a member of the Ziziphoid group within Rhamnaceae, and there is weak support for a sister relationship to a clade consisting of the Australian Pomaderreae and North America *Ceanothus* (Richardson *et al.* 2000).

***Cliffortia* (Rosaceae)** Non-CFR species that have been sampled in phylogenetic analysis are well embedded within the Cape group (Whitehouse 2002). The sister-group relationship between *Cliffortia* and the North Temperate *Sanguisorba* is weakly supported; this clade is sister to the South Temperate clade of *Acaena* and the Andean *Polylepis* (Helfgott *et al.* 2000). This indicates weak support for a European or a general Southern Hemisphere relationship.

Gnideae (Thymelaeaceae) This tribe (van der Bank *et al.* 2003) centered in the Cape, could well be a Cape clade. Species-level relationships in the clade are not yet known, therefore it cannot be determined whether the basal elements of the clade are found in the CFR. The nodes below Gnideae are poorly resolved, and involve taxa from Africa, Australasia, South America and North America. In this context the group is therefore biogeographically uninformative.

REFERENCES CITED

Adamson, R. S. 1958 The Cape as an ancient African flora. *The Advancement of Science* **58**, 1-10.

Axelrod, D. I. & Raven, P. H. 1978 Late Cretaceous and Tertiary vegetation history of Africa. In *Biogeography and ecology of southern Africa* (ed. M. J. A. Werger), pp. 79-130. The Hague: Junk.

Backlund, M., Oxelman, B. & Bremer, B. 2000 Phylogenetic relationships within the Gentianales based on *NDHF* and *rbcL* sequences, with particular reference to the Loganiaceae. *American Journal of Botany* **87**, 1029-1043.

Bakker, F. T., Hellbrügge, D., Culham, A. & Gibby, M. 1998 Phylogenetic relationships within *Pelargonium* sect. *Peristera (Geraniaceae)*, inferred from nrDNA and cpDNA sequence comparisons. *Plant Systematics and Evolution* **211**, 273-287.

Barker, N. P., Morton, C. M. & Linder, H. P. 2000 The Danthonieae: generic composition and relationships. In *Grasses: systematics and evolution* (ed. J. Everett), pp. 221-230. Melbourne: CSIRO.

Bolus, H. 1886 *Sketch of the flora of South Africa*. Cape Town: Richards and Sons.

Bradford, J. C. 2002 Molecular phylogenetics and morphological evolution in Cunonieae (Cunoniaceae). *Annals of the Missouri Botanical Garden* **89**, 491-503.

Bremer, B., Bremer, K., Heidari, N., Erixon, P., Olmstead, R. G., Anderberg, A. A., Kallersjo, M. & Barkhordarian, E. 2002 Phylogenetics of asterids based on 3 coding and 3 non-coding chloroplast DNA markers and the utility of non-coding DNA at higher taxonomic levels. *Molecular Phylogenetics and Evolution* **24**, 274-301.

Bremer, K. 2002 Gondwanan evolution of the grass alliance of families (Poales). *Evolution* **56**, 1374-1387.

Cooper, A., Lalueza-Fox, C., Anderson, S., Rambaut, A., Austin, J. & Ward, R. 2001 Complete mitochondrial genome sequences of two extinct moas clarify ratite evolution. *Nature* **409**, 704-707.

Cowling, R. M. 1987 Fire and its role in coexistence and speciation in Gondwanan shrublands. *South African Journal of Science* **83**, 106-112.

Cowling, R. M. (ed.) 1992 *The ecology of fynbos. Nutrients, fire and diversity*. Cape Town: Oxford University Press.

Cowling, R. M. & Pressey, R. L. 2003 Introduction to systematic conservation planning in the Cape Floristic Region. *Biological Conservation* **112**, 1-13.

Cowling, R. M., Rundel, P. W., Lamont, B. B., Arroyo, M. K. & Arianoutsou, M. 1996 Plant diversity in Mediterranean-climate regions. *Trends in Ecology and Evolution* **11**, 362-366.

Crisp, M., Cook, L. & Steane, D. 2004 Radiation of the Australian flora: what can comparisons of molecular phylogenies across multiple taxa tell us about the evolution of diversity in present-day communities? *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* **359**, 1551-1571.

Crisp, M. D., Gilmore, S. & Van Wyk, B.-E. 2000 Molecular phylogeny of the genistoid tribes of papilionoid legumes. In *Advances in Legume Systematics 9* (ed. A. Bruneau), pp. 249-276. Kew: Royal Botanic Gardens.

Crisp, M. D., West, J. G. & Linder, H. P. 1999 Biogeography of the terrestrial flora. In *Flora of Australia*, vol. 1, Introducion (ed. A. E. Orchard), pp. 321-367. Melbourne: ABRS/CSIRO Australia.

Croizat, L. 1958 *Panbiogeography*. Caracas: Published by the Author.

Douzery, E. J. P., Pridgeon, A. M., Kores, P., Linder, H. P., Kurzweil, H. & Chase, M. W. 1999 Molecular phylogenetics of Diseae (Orchidaceae): a contribution from nuclear ribosomal ITS sequences. *American Journal of Botany* **86**, 887-899.

Fan, C. & Xiang, Q. Y. 2003 Phylogenetic analyses of Cornales based on 26S rRNA and combined 26 rDNA-matK-rbcL sequence data. *American Journal of Botany* **90**, 1357-1372.
Francis, J. E. & Coffin, M. F. 1992 Cretaceous fossil wood from the Raggatt Basin, Southern Kerguelen Plateau (Site 50). In *Proceedings of the Ocean Drilling Program, Scientific Results*, vol. 120 (ed. E. M. Barbu), pp. 273-280: College Station, TX (Ocean Drilling Program).
Germishuizen, G. & Meyer, N. L. 2003 *Plants of southern Africa: an annotated checklist*. Pretoria: National Botanical Institute.
Givnish, T. J., Evans, T. M., Pires, J. C. & Systma, K. J. 1999 Polyphyly and convergent morphological evolution in Commelinales and Commelinidae: evidence from rbcL sequence data. *Molecular Phylogenetics and Evolution* **12**, 360-385.
Goldblatt, P. 1978 An analysis of the flora of Southern Africa: its characteristics, relationships, and origins. *Annals of the Missouri Botanical Garden* **65**, 369-436.
Goldblatt, P. 1990 Phylogeny and classification of Iridaceae. *Annals of the Missouri Botanical Garden* **77**, 607-627.
Goldblatt, P. & Le Thomas, A. 1997 Palynology, phylogenetic reconstruction, and classification of the Afro-Madagascan genus *Aristea* (Iridaceae). *Annals of the Missouri Botanical Garden* **84**, 263-284.
Goldblatt, P., Le Thomas, A. & Suarez-cervera, M. 2004 Phylogeny of the Afro-Madagascan *Aristea* (Iridaceae) revisited in the light of new data on pollen morphology. *Botanical Journal of the Linnean Society* **144**, 41-68.
Goldblatt, P. & Manning, J. 2000 *Cape Plants. A conspectus of the Cape flora of South Africa*. Pretoria: National Botanical Institute.
Goldblatt, P. & Manning, J. C. 2002 Plant diversity of the Cape Region of southern Africa. *Annals of the Missouri Botanical Garden* **89**, 281-302.
Goldblatt, P., Savolainen, V., Porteous, O., Sostaric, I., Powell, M., Reeves, G., Manning, J. C., Barraclough, T. G. & Chase, M. W. 2002 Radiation in the Cape flora and the phylogeny of peacock irises *Moraea* (Iridaceae) based on four plastid DNA regions. *Molecular Phylogenetics and Evolution* **25**, 341-360.
Gordon-Gray, K. D. 1995 *Cyperaceae in Natal*. Pretoria: National Botanical Institute.
Graur, D. & Martin, W. 2004 Reading the entrails of chickens: molecular timescales of evolution and the illusion of precision. *Trends in Genetics* **20**, 80-86.
Haines, R. W. & Lye, K. A. 1983 *The sedges and rushes of East Africa: a flora of the families Juncaceae and Cyperaceae in East Africa - with particular reference to Uganda*. Nairobi: East African Natural History Society.
Hedberg, O. 1965 Afroalpine flora elements. *Webbia* **19**, 519-529.
Helfgott, D. M., Francisco-Ortegam, J., Santos-Guerra, A., Jansen, R. K. & Simpson, B. B. 2000 Biogeography and breeding system evolution of the woody *Bencomia* alliance (Rosaceae) in Macaronesia based on ITS sequence data. *Systematic Botany* **25**, 82-97.
Hilger, H. H. & Böhle, U.-R. 2000 *Pontechium*: a new genus distinct from *Echium* and *Lobostemon* (Boraginaceae). *Taxon* **49**, 737-746.
Hnatiuk, R. J. 1990 *Census of Australian vascular plants*. Australian Flora and Fauna Series Number 11. Canberra: Australian Government Publishing Services.
Hooker, J. D. 1860 *Introductory Essay. The botany of the Antarctic Voyage. Part III. Flora Tasmaniae, Volume 1*. London: Lovell Reeve.
Hoot, S. B. & Douglas, A. W. 1998 Phylogeny of the Proteaceae based on atpB and atpB-rbcL intergenic spacer region sequences. *Australian Systematic Botany* **11**, 301-320.
Hopper, S. D., Fay, M. F., Rossetto, M. & Chase, M. W. 1999 A molecular phylogenetic analysis of the bloodroot and kangoroo paw family, Haemodoraceae: taxonomic,

biogeographic and conservation implications. *Botanical Journal of the Linnean Society* **131**, 285-299.
Johnson, L. A. S. & Briggs, B. G. 1975 On the Proteaceae - the evolution and classification of a southern family. *Botanical Journal of the Linnean Society* **70**, 83-182.
Johnson, L. A. S. & Briggs, B. G. 1981 Three old southern families - Myrtaceae, Proteaceae and Restionaceae. In *Ecological biogeography of Australia* (ed. A. Keast), pp. 429-469. Utrecht: Junk.
Johnson, S. D. 1995 Pollination, adaptation and speciation models in the Cape flora of South Africa. *Taxon* **45**, 59-66.
Jürgens, N. 1991 A new approach to the Namib Region. *Vegetatio* **97**, 21-38.
Jürgens, N. 1997 Floristic biodiversity and history of African arid regions. *Biodiversity and Conservation* **6**, 495-514.
Klak, C., Hedderson, T. A. & Linder, H. P. 2003 A molecular systematic study of the *Lampranthus* group (Aizoaceae) based on the chloroplast *trnL-trnF* and nuclear ITS and *5S* NTS sequence data. *Systematic Botany* **28**, 70-85.
Kron, K. A., Judd, W. S., Stevens, P. F., Crayn, D. M., Anderberg, A. A., Gadek, P. A., Quinn, C. J. & Luteyn, J. L. 2002 Phylogenetic classification of Ericaceae: molecular and morphological evidence. *The Botanical Review* **68**, 335–423.
Levyns, M. R. 1938 Some evidence bearing on the past history of the Cape flora. *Transactions of the Royal Society of South Africa.*, 401-424.
Levyns, M. R. 1952 Clues to the past in the Cape flora of today. *South African Journal of Science* **49**, 155-164.
Levyns, M. R. 1954 The genus *Muraltia*. *Journal of South African Botany, Supplementary volume* **2**, 1-247.
Levyns, M. R. 1958 The phytogeography of members of Proteaceae in Africa. *Journal of South African Botany* **24**, 1-9.
Levyns, M. R. 1962 Possible antarctic elements in the South African Flora. *South African Journal of Science* **58**, 237-241.
Levyns, M. R. 1964a Presidential address, migrations and origin of the Cape flora. *Transactions of the Royal Society of South Africa* **37**, 85-107.
Levyns, M. R. 1964b Presidential address. Migrations and origin of the Cape flora. *Trans. Roy. Soc. S. Africa* **37**, 85-107.
Linder, H. P. 1985 Gene flow, speciation, and species diversity patterns in a species-rich area: the Cape Flora. In *Species and speciation*, vol. 4 (ed. E. S. Vrba), pp. 53-57. Pretoria: Transvaal Museum.
Linder, H. P. 1987 The evolutionary history of the Poales/Restionales - a hypothesis. *Kew Bull.* **42**, 297-318.
Linder, H. P. 1994 Afrotemperate phytogeography: implications of cladistic biogeographical analysis. In *Proceedings of the XIIIth Plenary Meeting AETFAT, Malawi.*, vol. 2 (ed. A. C. Chikuni), pp. 913-930. Zomba: National Herbarium and Botanic Gardens, Malawi.
Linder, H. P. 2003 The radiation of the Cape flora, southern Africa. *Biological Reviews* **78**, 597-638.
Linder, H. P., Briggs, B. G. & Johnson, L. A. S. 2000 Restionaceae - a morphological phylogeny. In *Systematics and evolution of monocots - Vol. 1 of Proceedings of the Second International Conference on the Comparative Biology of the Monocots, Sydney, September 1998.* (ed. D. A. Morrison), pp. 653-660. Melbourne: CSIRO.
Linder, H. P., Eldenäs, P. & Briggs, B. G. 2003 Contrasting patterns of radiation in African and Australian Restionaceae. *Evolution* **57**, 2688-2702.

Linder, H. P., Hardy, C. R. & Rutschmann, F. 2005 Taxon sampling effects in molecular clock dating: an example from the African Restionaceae. *Molecular Phylogenetics and Evolution* **35**, 569-582.
Linder, H. P., Meadows, M. E. & Cowling, R. M. 1992 History of the Cape flora. In *The ecology of fynbos: nutrients, fire and diversity* (ed. R. M. Cowling), pp. 113-134. Cape Town: Oxford University Press.
Mabberley, D. J. 1997 *The plant book*. Cambridge: Cambridge University Press.
Marloth, R. 1908 Das Kapland, insonderheit das Reich der Kapflora, das Waldgebiet und die Karroo, pflanzengeographisch dargestelt. In *Wissenschaffliche Ergebnisse der deutschen Tiefsee-Expedition auf dem Dampfer "Valdivia" 1898-1899*, vol. 2, part 3 (ed. C. Chun), pp. 1-427. Jena: Gustav Fischer.
McGlone, M. S., Duncan, R. P. & Heenan, P. B. 2001 Endemism, species selection and the origin and distribution of the vascular plant flora of New Zealand. *Journal of Biogeography* **28**, 199-216.
McGuire, A. F. & Kron, K. A. 2005 Phylogenetic relationships of European and African Ericas. *International Journal of Plant Science* **166**, 311-318.
McLoughlin, S. 2001 The breakup history of Gondwana and its impact on pre-Cenozoic floristic provincialism. *Australian Journal of Botany* **49**, 271-300.
Mohr, B. A. R. & Gee, C. T. 1992 An early Albian palynoflora from the Kerguelen Plateau, Southern Indian Ocean (Leg 120). In *Proceedings of the Ocean Drilling Program, Scientific Results*, vol. 120 (ed. E. M. Barbu), pp. 255-271: College Station, TX (Ocean Drilling Program).
Muasya, A. M., Bruhl, J. J., Simpson, D. A., Culham, A. & Chase, M. W. 2000 Suprageneric phylogeny of Cyperaceae: A combined analysis. In *Monocots: Systematic and evolution* (ed. K. L. Wilson & D. A. Morrison), pp. 593-601. Melbourne: CSIRO.
Muasya, A. M., Simpson, D. A., Chase, M. W. & Culham, A. 2001 A phylogeny of *Isolepis* (Cyperaceae) inferred using plastid rbcL and trnL-F sequence data. *Systematic Botany* **26**, 342-353.
Oliver, E. G. H. 1991 The Ericoideae (Ericaceae) - a review. *Contributions from the Bolus Herbarium* **13**, 158-208.
Parrish, J. T. 1993 The palaeogeography of the opening South Atlantic. In *The Africa-South America connection* (ed. R. Lavocat), pp. 8-27. Oxford: Clarendon Press.
Persson, C. 2001 Phylogenetic relationships in *Polygalaceae* based on plastid DNA sequences from the *trnL-F* region. *Taxon* **50**, 763-779.
Plunkett, G. M., Soltis, D. E. & Soltis, P. S. 1997 Clarification of the relationship between Apiaceae and Araliaceae based on *mat*K and *rbc*L sequence data. *American Journal of Botany* **84**, 565-580.
Price, R. A. & Palmer, J. D. 1993 Phylogenetic relationships of the Geraniaceae and Geraniales from rbcL sequence comparisons. *Annals of the Missouri Botanical Garden* **80**, 661-671.
Reeves, G., Chase, M. W., Goldblatt, P., Rudall, P. J., Fay, M. F., Cox, A. V., Lejeune, B. & Souza-Chies, T. 2001 Molecular systematics of Iridaceae: Evidence from four plastid DNA regions. *American Journal of Botany* **88**, 2074-2087.
Retief, E. & Van Wyk, A. E. 1998 The genus *Echium* (Boraginaceae) in southern Africa. *Bothalia* **28**, 167-177.
Richardson, J. E., Fay, M. F., Cronk, Q. C. B., Bowman, D. & Chase, M. W. 2000 A phylogenetic analysis of Rhamnaceae using *rbcL* and *trnL-F* plastid DNA sequences. *American Journal of Botany* **87**, 1309-1324.
Richardson, J. E., Weitz, F. M., Fay, M. F., Cronk, Q. C. B., Linder, H. P., Reeves, G. & Chase, M. W. 2001a Phylogenetic analysis of *Phylica* L. with an emphasis on island

species: evidence from plastid *trnL-F* DNA and nuclear internal transcribed spacer (ribosomal DNA) sequences. *Taxon* **50**, 405-427.
Richardson, J. E., Weitz, F. M., Fay, M. F., Cronk, Q. C. B., Linder, H. P., Reeves, G. & Chase, M. W. 2001b Rapid and recent origin of species richness in the Cape flora of South Africa. *Nature* **412**, 181-183.
Rivadavia, F., Kondo, K., Kato, M. & Hasebe, M. 2003 Phylogeny of the sundews, *Drosera* (Droseraceae), based on chloroplast *rbc*L and nuclear 18S ribosomal DNA sequences. *American Journal of Botany* **90**, 123-130.
Sampson, S. D., Witmer, L. M., Forster, C. A., Krause, D. W., O'Connor, P. M., Dodson, P. & Ravoavy, F. 1998 Predatory dinosaur remains from Madagascar: implications for the Cretaceous biogeography of Gondwana. *Science* **280**, 1048-1051.
Sanderson, M. J. 1997 A nonparametric approach to estimating divergence times in the absence of rate constancy. *Molecular Biology and Evolution* **14**, 1218-1231.
Schlich, R. & Wise, S. W. J. 1992 The geologic and tectonic evolution of the Kerguelen Plateau: and introduction to the scientific results of Leg 120. In *Proceedings of the Ocean Drilling Program, Scientific Results*, vol. 120 (ed. E. M. Barbu), pp. 5-30: College Station, TX (Ocean Drilling Program).
Scholtz, A. 1985 The palynology of the upper lacustrine sediments of the Arnot Pipe, Banke, Namaqualand. *Annals of the South African Museum* **95**, 1-109.
Schönenberger, J. & Conti, E. 2003 Molecular phylogeny and floral evolution of Penaeaceae, Oliniaceae, Rhynchocalycaceae, and Alzateaceae (Myrtales). *American Journal of Botany* **90**, 293-309.
Schrire, B. D., Lavin, M., Barker, N. P., Cortes-Burns, H., von Senger, I. & Kim, J. 2003 Towards a phylogeny of *Indigofera* (Leguminosae-Papilionoideae): indentification of major clades and relative ages. In *Advances in Legume Systematics, part 10, higher level systematics* (ed. A. Bruneau), pp. 269-302. Kew: Royal Botanic Gardens.
Scotese, C. R. 1997 *Paleogeographic Atlas, PALEOMAP Progress Report 90-0497*. Arlington, Texas: Department of Geology, University of Texas at Arlington.
Scott, J. K. & Panetta, F. D. 1993 Predicting the Australian weed status of southern African plants. *Journal of Biogeography* **20**, 87-93.
Sosa, V. & Chase, M. W. 2003 Phylogenetics of Crossosomataceae based on *rbcL* sequence data. *Systematic Botany* **28**, 96-105.
Takhtajan, A. 1986 *Floristic regions of the world*: University of California Press, Berkeley.
Thorne, R. F. 1973 Floristic relationships between tropical Africa and tropical America. In *Tropical Forest Ecosystems in Africa and South America: a comparative Review.* (ed. W. D. Duckworth), pp. 27-47. Washington: Smithsonian Institution Press.
van der Bank, M., Chase, M. W., Van Wyk, B.-E., Fay, M. F., van der Bank, F. H., Reeves, G. & Hulme, A. 2002 Systematics of the tribe Podalyrieae (Fabaceae) based on DNA, morphological and chemical data. *Botanical Journal of the Linnean Society* **139**, 159-170.
van der Bank, M., Fay, M. F. & Chase, M. W. 2003 Molecular phylogenetics of Thymelaeaceae with particular referec to African and Australian genera. *Taxon* **51**, 329-339.
van der Walt, J. J. A. & Vorster, P. J. 1983 Phytogeography of *Pelargonium*. *Bothalia* **14**, 517-523.
van Wyk, B.-E. 1991 A synopsis of the genus Lotononis (Fabaceae:Crotalarieae). *Contributions from the Bolus Herbarium* **14**, 1-292.
Verboom, G. A., Linder, H. P. & Stock, W. D. 2003 Phylogenetics of the grass genus *Ehrharta* Thunb.: evidence for radiation in the summer-arid zone of the South African Cape. *Evolution* **57**, 1008-1021.

Vinnersten, A. & Reeves, G. 2003 Phylogenetic relationships within Colchicaceae. *American Journal of Botany* **90**, 1455-1462.
Wagner, W. L., Herbst, D. R. & Sohmer, S. H. 1990 *Manual of the flowering plants of Hawai`i*. Bishop Museum Special Publication. Honalulu: University of Hawaii and Bishop Museum Press.
Weimarck, H. 1933 Die Verbreitung einiger Afrikanisch-montanen Pflanzengruppen, I-II. *Svensk Botanisk Tidskrift* **27**, 400-419.
Weimarck, H. 1936 Die Verbreitung einiger Afrikanisch-montanen Pflanzengruppen, III-IV. *Svensk Botanisk Tidskrift* **30**, 36-56.
Whitehouse, C. M. 2002 Systematics of the genus *Cliffortia* L. Rosaceae. In *Department of Botany*, pp. 174. Cape Town: University of Cape Town.
Wikström, N., Savolainen, V. & Chase, M. W. 2001 Evolution of the angiosperms: calibrating the family tree. *Proceedings of the Royal Society of London Series B-Biological Sciences* **268**, 2211-2220.
Wild, H. 1964 The endemic species of the Chimanimani mountains and their significance. *Kirkia* **4**, 125-157.
Wild, H. 1968 Phytogeography in South Central Africa. *Kirkia* **6**, 197-222.
Winkworth, R. C., Wagstaff, S. J., Glenny, D. & Lockhart, P. J. 2002 Plant dispersal NEWS from New Zealand. *Trends in Ecology and Evolution* **17**, 514-520.
Wojciechowski, M. F. 2003 Reconstructing the phylogeny of legumes: and early 21st century perspective. In *Advances in Legume Systematics, part 10, Higher Level Systematics* (ed. A. Bruneau), pp. 5-35. Kew: Royal Botanic Gardens.
Zachos, J., Pagani, M., Sloan, L., Thomas, E. & Billups, K. 2001 Trends, rhythms, and aberrations in global climate 65 Ma to present. *Science* **292**, 686-693.

The Cape element in the Afrotemperate flora: from Cape to Cairo?

CHLOE GALLEY[1]*, BENNY BYTEBIER[2]*, DIRK U. BELLSTEDT[2] AND H. PETER LINDER[1]

[1]*Institute of Systematic Botany, University of Zurich, Zollikerstrasse 107, CH-8008 Zurich, Switzerland*

[2]*Department of Biochemistry, Stellenbosch University, Private Bag X1, 7602 Matieland, South Africa*

* This chapter was jointly written with Benny Bytebier from Stellenbosch University, with whom the methods and interpretation were developed.

Proceedings of the Royal Society B: Biological Sciences 2007, **274**: 535-543

ABSTRACT

The build up of biodiversity is the result of immigration and *in situ* speciation. We investigate these two processes for four lineages (*Disa*, Irideae p.p., the *Pentaschistis* clade and Restionaceae) that are widespread in the Afrotemperate flora. These four lineages may be representative of the numerous clades which are species rich in the Cape and also occur in the highlands of tropical Africa. It is as yet unclear in which direction the lineages spread. Three hypotheses have been proposed: (i) a tropical origin with a southward migration towards the Cape; (ii) a Cape origin with a northward migration into tropical Africa and (iii) vicariance. None of these hypotheses has been thoroughly tested. We reconstruct the historical biogeography of the four lineages using likelihood optimisation onto molecular phylogenies. We find that tropical taxa are nested within a predominantly Cape clade. There is unidirectional migration from the Cape into the Drakensberg and from there northwards into tropical Africa. The amount of *in situ* diversification differs between areas and clades. Dating estimates show that the migration into tropical East Africa has occurred in the last 17 million years, consistent with the Mio-Pliocene formation of the mountains in this area.

Keywords: historical biogeography, ancestral character reconstruction, phytogeography, molecular dating, Africa

INTRODUCTION

Local floras and faunas accumulate diversity by the recruitment of new lineages as well as by *in situ* speciation. The sourcing of lineages has long occupied biogeographers: historical biogeographers list the diverse "elements" for a biota (Wulff 1950), panbiogeographers assemble "tracks" showing the shared elements between biotas (Craw *et al.* 1999), and cladistic biogeographers compile sets of "components" or three-area statements (Nelson & Ladiges 1996; Humphries & Parenti 1999). The increasing availability of dated phylogenies has made it possible to understand how and when biomes were assembled (Crisp 2006). No region is isolated from immigration, consequently the relative roles of immigration and *in situ* diversification are more difficult to untangle for regions which share many lineages. Well sampled phylogenies are needed to determine whether an endemic species in a biota speciated locally or if it was recruited from a "source area".

Here we explore the phytogeographical patterns in the Afrotemperate region (Weimarck 1936; Wild 1964; Linder 1990). The region is an archipelago of isolated areas ranging from the highlands of Ethiopia in the northeast, to the southern tip of Africa, and to the Fouta Djalon in Guinea in the west (White 1978). It combines the Afromontane and Cape phytochoria of White (1983). These patches of temperate vegetation, often separated by thousands of kilometres, can be grouped into three centres of endemism: the Cape Region, the greater Drakensberg Range and the Afromontane Centre (Linder 1990). Although the floristic affinities between these centres have frequently been explored (Weimarck 1941; Hedberg 1965; Wild 1968; Killick 1978; White 1978; Linder 1990), the historical biogeography remains enigmatic.

Many of the very diverse Cape clades (Linder 2003) also occur in the Afromontane Region (Cowling 1983; Carbutt & Edwards 2002) although their species richness decreases to the west and the north. Their contribution to the floras of these regions is substantial: Hilliard & Burtt (1987) considered an estimated 22% of the genera recorded in the southern Drakensberg as "centred in the Cape Region", whilst Hedberg (1965) showed that 4% of Afroalpine flora elements form a "Cape element". These constitute part of the austral element in the Afrotemperate flora, and complement the boreal element which has more north temperate affinities.

Three main hypotheses for the origin and migration of these taxa have been postulated to explain their current distribution: (i) an origin in tropical Africa and migration through the

Afromontane Region southwards into the Cape (Levyns 1938; Levyns 1952; Levyns 1964) (ii) an origin in the Cape and migration northwards into tropical Africa (Linder 1994); (iii) vicariance, with the floras in each region representing relics from a once widespread African flora that has receded with climatic changes (Adamson 1958; Wild 1968; King 1978). Although the disjunctions are well documented and the historical processes have been discussed (Levyns 1952; Adamson 1958; Levyns 1962; Levyns 1964; Wild 1968; King 1978; Van Zinderen Bakker 1978), few authors have tested these hypotheses. Linder (1994) rejected a north to south migration on the basis of a cladistic biogeographical analysis of Disinae (Orchidaceae) and Griswold (1991) found it difficult to reconcile a Pleistocene vicariance scenario with the current distribution of Afromontane spiders. McGuire & Kron (2005) inferred a north to south migration for the Cape mega genus *Erica*, but did so on the basis of an under-sampled and poorly supported molecular phylogenetic tree.

Here we reconstruct ancestral distributions using likelihood optimisation on phylogenetic hypotheses, for four clades with a classical Afrotemperate distribution pattern, with the majority of their species restricted to the Cape. We test the directionality of migrations through Africa and address the following questions:

- Is migration between the Cape and regions north of the Limpopo River direct, or do we infer movement via the Drakensberg?
- How does the temporal sequence and dating of the migration events relate to the geological history of Africa?
- Did taxa that are found outside the Cape speciate *in situ* or are they derived from separate migration events?

MATERIAL AND METHODS

Phylogenetic trees

The phylogenetic hypotheses for the four study groups and sequence data for the dating were derived from the following sources: *Disa*, cpDNA and nrDNA (Bytebier *et al.* 2006); Irideae p.p., cpDNA (Goldblatt *et al.* 2002, figure 3); the *Pentaschistis* clade, cpDNA (Galley & Linder 2007, figure 3); the African Restionaceae, cpDNA (Hardy *et al.* submitted). In the cases of *Disa* and the Restionaceae, the topology of the tree with the highest likelihood score from the set of Bayesian trees was used.

Age estimations

Trees were made ultrametric and the ages of the disjunctions in the four study groups were estimated using a Bayesian relaxed clock (Renner 2005; Rutschmann 2006), as implemented in Multidivtime (Thorne & Kishino 2002). There are no fossils available for the study groups so the crown node of each study group was constrained with a calibration point derived from a separate 'global analysis', which had four fossil calibration points. Details of the multidivtime analysis and the global analysis can be found in Appendix 1.

Areas and taxon scoring

Taxa were scored as present or absent for six regions, shown in Figure 1: The Greater Cape Floristic Region (hereafter referred to as the "Cape") (Jürgens 1991; Born *et al.* 2006); the Drakensberg Range (DR) comprising the Drakensberg escarpment (Partridge & Maud 1987), upland areas south to Elliot and north to Tzaneen (Carbutt & Edwards 2004); Zimbabwe Overlap Region (ZOR) between the Limpopo and Zambesi rivers; South Central African centre (SCA) including Mount Mulanje, the Nyika plateau, and the southern Tanzanian highlands; 'Eastern Africa' (EA) including the central and east African uplands, as well as the Ethiopian plateau; and 'Western Africa' (WA) comprising the Cameroon highlands and the uplands westward to the Fouta Djalon. Other areas were also scored where applicable (Réunion, Madagascar, the Mediterranean and Amsterdam Island / St. Paul Island). Widespread taxa were coded as present in all relevant areas. An exception to this is *Pentaschistis natalensis* for which the three accessions (from Natal, Madagascar and South-Central Africa) did not form a clade, consequently each accession was coded according to the area in which it was collected.

Reconstruction of biogeographical history

The distribution ranges of ancestral nodes were reconstructed using likelihood optimisation as implemented in Mesquite 1.1 (Maddison & Maddison 2006), using the rate-corrected branch lengths. Each node was optimised as present versus absent for each of the six areas. A threshold value of 2 log likelihood (lnL) units was used to indicate statistical significance for

the ancestral state optimisation of each node (Mooers & Schluter 1999; Maddison & Maddison 2006). We compared the lnL scores of a two-rate (forward and backward rates independent) and a one-rate (forward and backward rates constrained to be equal) model for each character, for each taxon. The accuracy of parameter estimation depends on the amount of data available and the frequency of the minority character state, as well as model complexity (Mooers & Schluter 1999). All taxa had several characters for which the use of the two-rate model did not result in a significantly improved fit (sometimes a worse fit was obtained) and we therefore used the one-rate model for optimisation. This handles trees with few transitions and an imbalance of character states better than the two-rate model (Schluter *et al.* 1997; Mooers & Schluter 1999).

To infer the ancestral distribution of a node, the optimisation for each area was taken into account separately. The node was optimised as the area which was significantly 'present' at that node. In most cases the node was optimised as 'absent' for all other areas and was thus optimised unambiguously. In a number of cases a node was significantly optimised to more than one area. In nine cases, a node was optimised as absent to all areas except one, for which support was not significant but above 0.75 proportional likelihood (see Fig. 2, also Figs. 1 and 2 of Appendix 2). In these cases we assigned this area to the node. Nodes that were not assigned any area were omitted from further calculations.

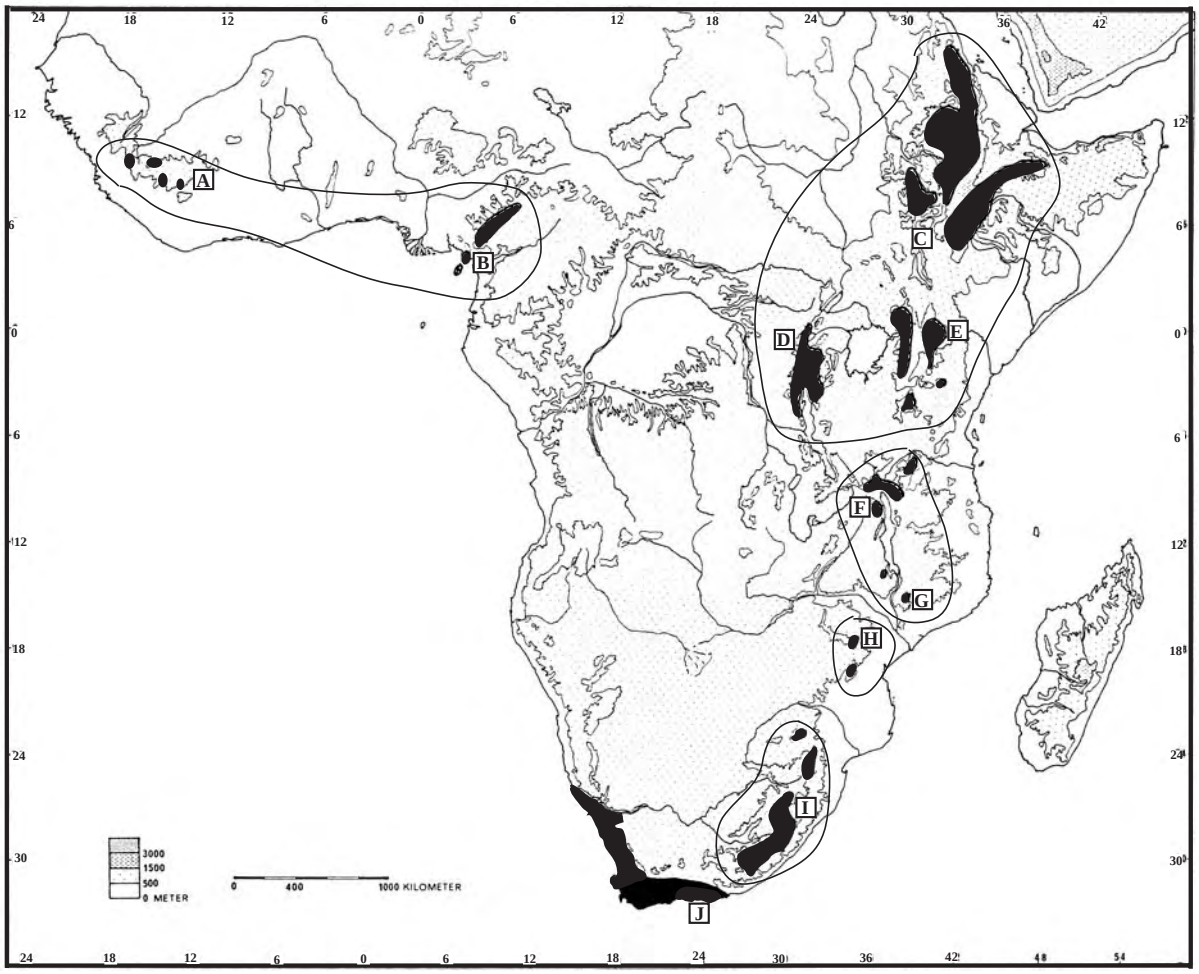


Figure 1. Map indicating areas used in analysis. 'Western Africa' including: (A) the Fouta Djalon in Guinea and (B) Mount Cameroon; 'Eastern Africa' including: (C) Ethiopian highlands, (D) Virunga and Rwenzori mountains and (E) north Tanzanian / west and central Kenyan / east Ugandan mountains; 'South Central African centre' including: (F) Nyika Plateau and southern Tanzanian highlands and (G) Mount Mulanje; 'Zimbabwe Overlap Region' including: (H) Chimanimani Mountains and Nyanga Plateau; the 'Drakensberg Range' (I); and the Greater Cape Floristic Region (J). Areas north of the Limpopo from White (1978). Map adapted from (Linder 1990).

A migration event on a branch was counted when the daughter node optimised to a different area than that of the parent node. Three types of migration were recognised: range expansion, when a node or taxon is present in the same area as its parent node, but occurs in an additional area; vicariance, when a parent node is optimised to two areas but the two daughter nodes each to only one of these; and dispersal, when the parent and daughter nodes optimise to different areas.

Calculations

We calculated the age of the dispersal events by estimating the age of the ancestral node of the branch which has the change in distribution. This assumes that the migration accompanied speciation. The same method was used to date range expansions (migration without accompanied speciation). Since no speciation accompanied range expansions, they cannot be dated precisely; the date could be any time between the node subtending the taxon, and the present. This approach therefore estimates a maximum age.

Migration is a function of movement from an area plus persistence, the former of which is related to the number of species in the source area. This needs to be taken into account to test for unidirectionality of migration, and we divided the number of migration events by the number of (sampled) species in the source area.

RESULTS

The ancestral node of each of the four clades (*Disa*, Irideae p.p., the *Pentaschistis* clade and Restionaceae) optimises to the Cape and to no other area. ~ 94% of the nodes in the four clades can be optimised unambiguously. Most of these optimise to single areas (see Fig. 1 and Figs. 1, 2 and 3 of Appendix 2) and in two clades (Irideae p.p. and *Disa*) there are four nodes which optimise unambiguously to multiple areas. A minimum of 31 dispersal events have been documented (see Table 1). Where range expansion has occurred without speciation, the precise route of migration cannot be known from a species-level study. Only unambiguous migrations are considered further, but detailed descriptions of the optimisations and migrations inferred for each clade are given in the Appendix 2. In all clades most dispersal events out of the Cape are to the DR (five events in *Disa*, three events in Irideae p.p., five events in the *Pentaschistis* clade and five or six events in Restionaceae), although there are two dispersals (in Irideae p.p.) directly to areas north of the Limpopo River. From the DR there are 12 events to north of the Limpopo River, meaning that the predominant source of the flora north of the Limpopo River is the DR, rather than the Cape (see Fig. 3). There are only two dispersals into the Cape from the DR (in *Disa*), demonstrating that most of the species diversity in the Cape is derived from a single lineage for each clade. In *Disa* and Irideae p.p. there has been diversification in the DR but in the *Pentaschistis* clade and Restionaceae all species in the DR have their sister species in the Cape, indicating that they have migrated into the region. *Disa* and Irideae p.p. have species sampled in the ZOR. With one exception in the Irideae p.p., all of these species have their sister species in other areas, indicating that the rate

of local diversification has also been very low there. The migration events from the Cape to the DR are more frequent than in the opposite direction, even if the number of taxa in the source area is taken into account (Table 2, Wilcoxon sign Ranks Test: Cape to DR vs. DR to Cape: P = 0.068).

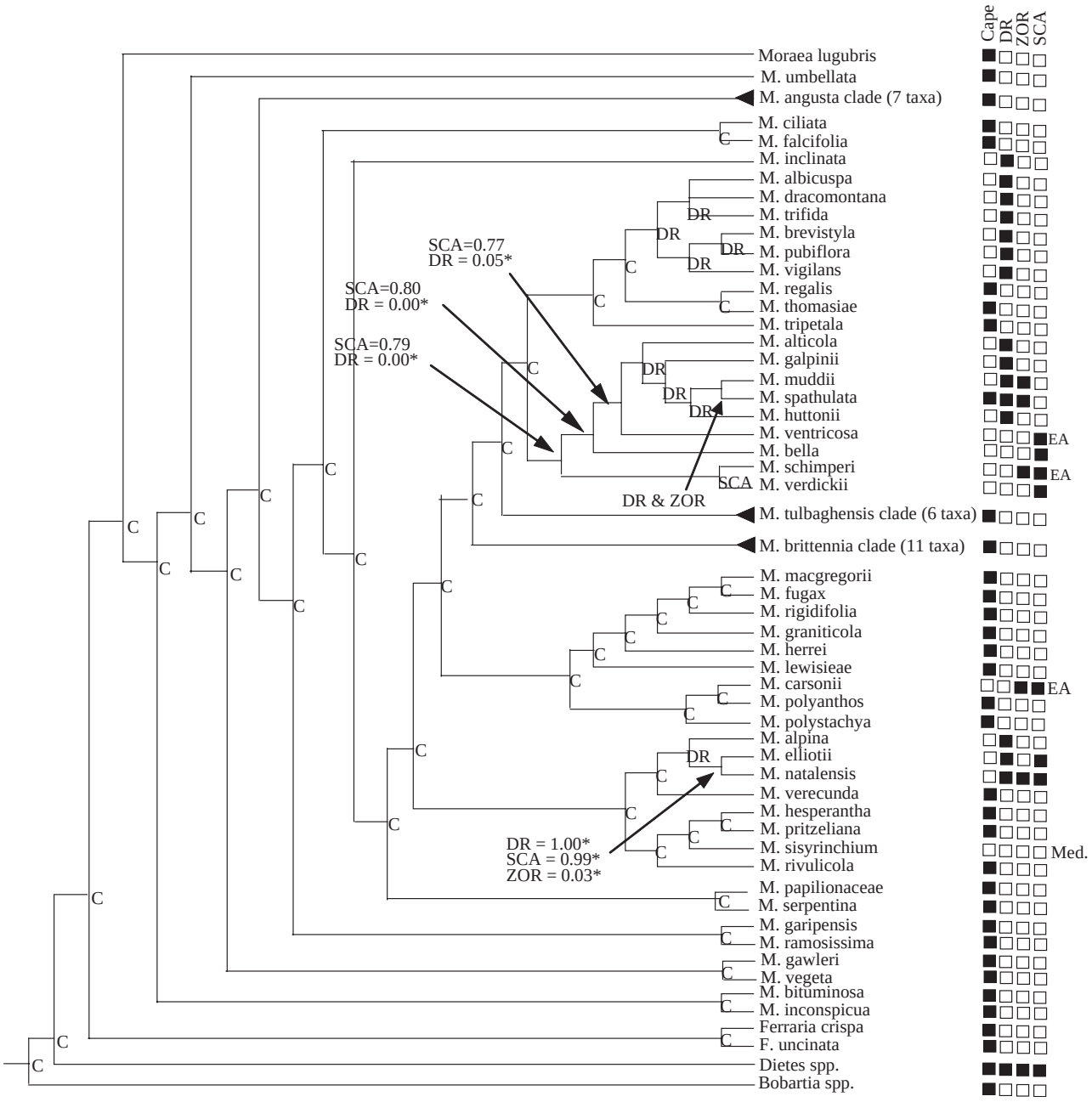


Figure 2. Optimisation of ancestral node distribution for the Irideae p.p. including proportional likelihoods of areas for nodes that do not optimise unambiguously. Area: C, Cape; DR, Drakensberg Range; ZOR, Zimbabwe Overlap Region; SCA, South-Central Africa; EA, East Africa; Med., Mediterranean.

The age estimates for the migration events area shown in Table 1. Where a migration could have occurred on either of two adjacent branches (because an intermediate node could not be unambiguously optimised), we report the range of the age estimates and the Credibility Interval (CrI). In the Irideae p.p. and *Disa* especially, there have been frequent range expansion without lineage diversification and the ages given are therefore maximum estimates (represented by "*" in Table 1).

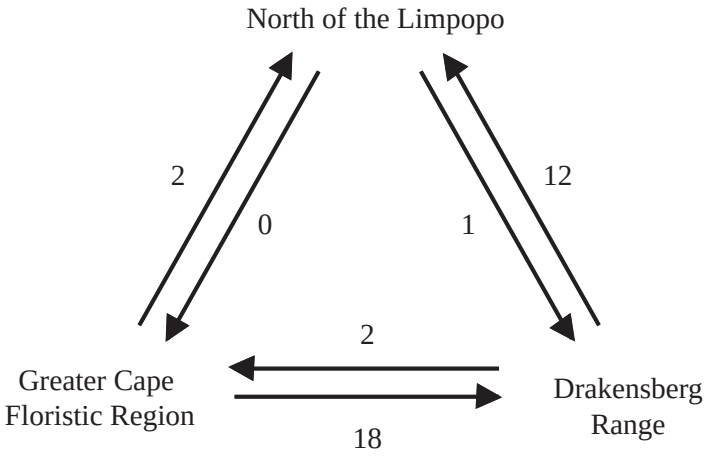


Figure 3. Diagram showing the number of migration events between the Greater Cape Floristic Region, the Drakensberg Range and north of the Limpopo River. Unambiguous migrations only were considered.

Table 1. Summary table of migrations inferred from ancestral state reconstruction, showing the age estimation of migration events. * = migration without speciation therefore the event may be between the date range given and the present. Where a range of nodes and thus age estimates is used, the credibility interval (CrI) shown represents the upper and lower extremes.

taxon	**from where**	**to where**	**age estimate (mean)**	**95% CrI**
Moraea albicuspa clade	Cape	DR	9.11	4.06, 16.37
M. inclinata	Cape	DR	25.65	17.25, 34.80
M. alpina clade	Cape	DR	18.33	11.06, 26.84
M. verdickii grade	Cape	SCA	14.98	8.19, 23.32
M. sisyrinchium	Cape	Mediterranean	13.56	7.04, 21.80
M. alticola clade	SCA	DR	8.26	3.68, 14.80
M. carsonii	Cape	ZOR, SCA, EA	7.69	2.87, 15.20
M. spathulata / *M. muddii* clade	DR	ZOR	4.68	1.50, 9.69
M. spathulata	DR, ZOR	Cape	2.73*	0.28, 6.95
Dietes	Cape	EA, SCA, ZOR, DR	51.45*	46.05, 54.78
M. schimperi	SCA	ZOR, EA	10.07*	4.59, 17.31
M. natalensis/ M. elliotii clade	DR	SCA	12.03*	5.78, 20.15
M. natalensis	DR, SCA	ZOR	4.28*	0.58, 10.43
Disa tripetaloides	Cape	DR	1.37*	0.14, 3.50
D. bracteata	Cape	DR	2.71*	1.16, 5.01
D. sagittalis and D. zimbabweensis	Cape	DR, ZOR	15.19	11.30, 19.44
D. lugens var. lugens	Cape	DR	1.7*	0.56, 3.65
D. cephalotes clade	Cape	DR	8.87	3.53, 12.92
clade 'z'	Cape	DR	17.88 to 15.39	21.41, 11.91

D. baurii	Cape	DR, ZOR, SCA	5.67	2.91, 9.30
D. brevicornis	Cape	DR, ZOR, SCA	0.82*	0.08, 1.67
D. cornuta	Cape	DR, ZOR	6.52*	3.84, 10.07
D. borbonica	Cape	Reunion Island	1.57	0.82, 1.99
D. patula var. transvaalensis	DR	ZOR	0.60*	0.03, 1.88
D. woodii	DR	ZOR	1.32*	0.18, 3.19
D. rhodantha	DR	ZOR	1.32*	0.18, 3.19
D. versicolor	DR	ZOR, SCA	0.61*	0.03, 1.91
D. saxicola	DR	ZOR, SCA	5.43*	2.69, 9.05
D. fragrans ssp. fragrans	DR	ZOR, SCA	4.87*	2.49, 8.18
D. miniata clade	DR	SCA	10.50 to 5.79	14.20, 3.00
D. perplexa clade	DR	SCA	10.61*	7.38, 14.28
D. chrysostachya	DR	Cape	2.67*	1.08, 5.14
D. buchenaviana	DR	Madagascar	8.23	5.36, 13.11
D. caffra	DR, SCA	Madagascar	2.38*	0.84, 4.73
D. aconitoides ssp. aconitoides	DR	Cape	9.05*	6.07, 12.67
D. aconitoides ssp. goetzeana	DR	EA	8.18	5.29, 11.70
D. similis	DR	SCA	8.18*	5.29, 11.70
D. hircicornis	DR, SCA	ZOR, EA, WA	2.38*	0.84, 4.73
D. perplexa	DR, SCA	ZOR, EA, WA	5.97*	3.38, 9.49
D. zombica	SCA	ZOR	2.45*	0.94, 4.83
D. miniata	SCA	ZOR	2.35*	0.78, 4.73
D. ornithantha	SCA	ZOR	2.30*	0.79, 4.61
D. erubescens ssp. erubescens	SCA	ZOR, EA	0.54*	0.03, 1.70
D. ochrostachya	SCA	EA, WA	2.35*	0.78, 4.73
D. stairsii	?	EA	5.79	3.00, 9.54
Pentaschistis tysonii	Cape	DR	9.95	7.75, 11.67
P. basutorum	Cape	DR	9.13	7.35, 11.37
P. aurea subsp. pilosogluma	Cape	DR	1.75	0.25, 3.79
	and /or DR			
P. exserta	Cape	DR	5.35	3.76, 7.67
P. chippindalliae	Cape	DR	3.77	2.44, 5.47
P. andringitrensis	Cape	Madagascar	8.03	6.24, 10.27
the summer clade	Cape	?	5.29 to 6.14	3.66, 8.32
P. insularis	?	Amsterdam Islands	0.44	0.02, 1.29
P. pictigluma clade	?	EA	1.04	0.47,1.88
P. natalensis	?	SCA / Madagascar	1.25 to 3.11*	0.20,4.61
Restio zuluensis	Cape	DR	5.87	2.28, 11.89
Ischyrolepis schoenoides	Cape	DR	4.66	1.39, 10.30
Calopsis paniculata	Cape	DR	17.31	9.95, 29.44
Re. Sejunctus	Cape	DR	5.35*	1.65, 11.59
Rhodocoma fruticosa	Cape	DR	1.25*	0.04, 4.45
Re. Galpinii	?	DR	16.64	8.61, 29.22
Re. mahonii and Re. mlanjiensis	?	EA and SCA, SCA	16.64	8.61, 29.22

Table 2. The migration rate for each clade, calculated as the number of migrations per sampled taxon in the source area.

	Cape to DR	**DR to Cape**
Disa	0.0732	0.0435
Irideae p.p.	0.0526	0
the *Pentaschistis* clade	0.0806	0
Restionaceae	0.0174	0

DISCUSSION

Methods

To reconstruct historical biogeography and biome assembly it is essential to know the source and direction of migrations (Crisp 2006). These can only be deduced if the ancestral areas of distribution are known. Dispersal-Vicariance Analysis (DIVA) (Ronquist 1997), a parsimony-based method, has commonly been used to infer ancestral areas, but we have instead used a likelihood-based method due to several advantages it has over DIVA. Perhaps the most important one is that it uses branch length information to calculate the probability of character state change, which on an ultrametric tree is directly related to time. Since older species will have had more time to disperse that younger species, this is an important parameter to take into account. A second advantage is that likelihood optimisation allows for quantification of the level of uncertainty in optimisation (Schluter *et al.* 1997). This is particularly important where there has been a lot of change in a short time (Schluter *et al.* 1997), or when optimising more basal nodes (Mooers & Schluter 1999).

We used presence-only coding and binary optimisation, rather than multistate coding and optimisation, since currently available likelihood optimisation software does not allow polymorphic character states at internal nodes, even if polymorphic states at the tips are accepted. Many of the species we investigated occur in more than one area (e.g. 19% of the *Disa* species occur in multiple areas) and we therefore assume that some internal nodes might also have had wider distributions. Such a scenario can only be reconstructed using binary optimisation (Hardy & Linder 2005), since internal nodes can be optimised as polymorphic with statistical significance.

Topological uncertainty was not dealt with directly, except in the case of Irideae p.p., where the phylogenetic hypothesis contained polytomies (Goldblatt *et al.* 2002). In *Disa* and the *Pentaschistis* clade there are several poorly supported nodes in areas of the topology

where several distributional changes probably took place. However, there was also ambiguity in the optimisation of these nodes (due both to this frequent change, and to the short branch lengths involved). These ambiguous nodes were omitted from further analysis.

Directionality and the sourcing of the Afromontane flora in tropical Africa

The MRCA of all four clades was unambiguously traced to the Cape. In total, 18 migrations from the Cape to the Drakensberg Range and 12 from the Drakensberg to the rest of the Afromontane Region north of the Limpopo River, are documented. There are two migrations from the Cape to north of the Limpopo River. Migration events in the opposite directions are rare (Fig. 3). This pattern refutes the hypothesis that north to south has been the prevailing direction of migration for taxa shared between the Cape and Afromontane floras (Levyns 1938; Levyns 1952; Levyns 1964; Axelrod & Raven 1978). Although neither the *Pentaschistis* clade nor *Disa* nor Irideae p.p. were mentioned explicitly, Levyns (1964) discussed many lineages that are similarly distributed within the Cape. Although she favoured a southern origin for a few taxa, such as Restionaceae and *Phylica*, the distribution of other lineages was postulated to be the result of north to south migration. This "southward migratory stream" was based mainly on the widespread but scattered distribution of more "primitive" relatives or members of the lineages in tropical Africa. These were seen as relics of a once more continuous vegetation. In contrast, more "advanced" members of the lineages were found in the south-western part of the Cape where, as "youthful endemics" they usually have a narrower distribution.

Although the 95% credibility intervals (CrI) of the age estimates are large, we date the migration of this flora to the tropical Afrotemperate regions to between 0.54 (0.02-1.80 CrI) to 10.0 (4.59-17.31 CrI) Myr ago (see Table 1). Such recent migrations into these areas are congruent with the recent formation of the uplands of tropical Africa, which dates to the Miocene, with further uplift in the Pliocene and Pleistocene (Grove 1983; Partridge *et al.* 1985). Palynological evidence suggests that *Podocarpus* and *Juniperus* were not in the Turkana Basin, northern Kenya, before 25 Myr ago (Vincens *et al.* 2006), and that they were present in Fort Ternan, Kenya, at around 14 Myr ago (Bonnefille *et al.* 2004). In contrast, the Cape Fold Mountains and the Lesotho highlands (within the Drakensberg Range) precede the evolution of the Angiosperms and were partially preserved through the early African erosion cycle (King 1963; Grove 1983; Partridge & Maud 1987; Partridge 1998). There was

important rejuvenation with two major periods of uplift in the early Miocene and the Pliocene especially in the Drakensberg (Partridge & Maud 1987; Partridge 1998; Partridge & Maud 2000). Stem lineages of members of the Cape clades date to the late Cretaceous and throughout the Tertiary (Galley & Linder 2006), consistent with the ancient Cape mountains. Migrations into the Drakensberg date to as early as 25.65 (17.25-34.80 CrI) Myr ago, and there is an increase in the number of events in the last 9 Myr, consistent with recent uplift in the area.

The Drakensberg Range in the spread of the Afrotemperate flora

The Drakensberg Range plays an important role as a 'stepping-stone' for plants between the Cape and the tropical Afrotemperate Region (Fig. 3). The close floristic affinity between the Cape and the Drakensberg Range is well known (Weimarck 1941; Killick 1963; Killick 1978; Hilliard & Burtt 1987; Carbutt & Edwards 2002). We demonstrate it to be the result of many migration events occurring over a wide time span, and largely in one direction. This unidirectional migration cannot be explained simply by the number of taxa in the source area.

The Drakensberg Range has been proposed to be the source of many of the Cape elements in the mountains of tropical Africa (Weimarck 1941). This has been demonstrated for *Aloe* (Holland 1978) and Coryciinae s.s. (Linder 1994). This stepping-stone role of the Drakensberg Range in the spread of species through the Afrotemperate region is well supported by our data (Fig. 3). Although there are a few exceptions (*Moraea carsonii* and the *M. verdickii* grade, plus potential additional cases in *Restio* and *Pentaschistis*), direct migration from the Cape to areas north of the Limpopo is not the norm. Furthermore, any extinction in the Drakensberg Range would mask an indirect route. The Drakensberg Range could also be the source of other Austral Afrotemperate taxa also represented in the Cape, such as *Satyrium* (Orchidaceae), *Kniphofia* and *Aloe* (Asphodelaceae).

Speciation outside of the Greater Cape Floristic Region

The Cape is known for its very high species richness (Levyns 1964; Goldblatt 1978; Goldblatt & Manning 2002; Linder 2003) concentrated in a relatively small number of clades (Levyns 1964; Goldblatt 1978; Linder 2003). It is unclear whether the high richness of the Cape

relative to the other regions is the result of a more rapid diversification rate, or simply of accumulation of species over a longer time period.

The wind-pollinated Restionaceae and *Pentaschistis* clades are represented by singleton species in the Drakensberg Range, meaning that there has been no local diversification (i.e. speciation has not exceeded extinction). This cannot be explained by a lack of time to speciate since some of these migration events are very old (Table 1), and in the *Pentaschistis* clade, for example, one Drakensberg species (*P. basutorum*) is sister to a clade of at least 48 Cape species. Since all but one Drakensberg species from both clades have been sampled, we would not expect the pattern to change much with increased sampling. The biotically pollinated *Disa* and Irideae p.p. show a different pattern. *Disa* has reached the Drakensberg Range at least ten times and includes two clades that have subsequently radiated in the region, resulting in 12 and 26 taxa respectively. Likewise Irideae p.p. has reached the Drakensberg at least six times and has speciated *in situ* resulting in three clades of three, five and seven taxa. The relative influence of pollinators and habitat diversity in the Cape and the Drakensberg Range may have played an important role in the origin of differences between these two sets of clades, and should be investigated.

While the Drakensberg Range represents a source area for the more northerly part of the Afrotemperate Region, the Zimbabwe Overlap Region acts more like a sink. *Disa* and Irideae p.p. reached the Zimbabwe Overlap Region at least 15 and four times respectively, but we document only one instance of local speciation. However, unlike *Pentaschistis* and Restionaceae in the Drakensberg Range, these migrations are on average amongst the youngest events (Table 1). Weimarck (1941) viewed the Inyangani Subcentre (=Zimbabwe Overlap Region) as a “relic”, but although the habitats in these areas may be old, many of the species are clearly relatively recent additions. It is possible that the small surface area of the uplands in this region (about 1,600 km^2, Timberlake & Muller 1994) may be linked to a higher probability of local extinction (Gaston 2003). A consequence would be that the contemporary taxa are relative newcomers to the area. *Disa* and Irideae p.p. in the Zimbabwe Overlap Region have been sourced from both the north (South Central Africa) and the south (the Cape or the Drakensberg Range), and this mixed sourcing of the flora is consistent with the suggestions of Weimarck (1941) and Van Wyk & Smith (2001). Four species of Irideae p.p., one of *Disa* and one of Restionaceae are endemic to the Zimbabwe Overlap Region but were not sampled here. Although this makes our figure an underestimation, including these would not alter our conclusions that this region has a low diversification rate and that there are multiple sources to its flora.

All four lineages have species in South Central and Eastern Africa. For three of these (Restionaceae, the *Pentaschistis* clade and *Disa*) we know that *in situ* speciation has contributed at least half of the species. One of the two species of Restionaceae evolved in South Central Africa. The five *Pentaschistis* taxa in Eastern Africa form a clade, showing a radiation from a single immigration to the area. *Disa* is represented by 45 species in South Central and Eastern Africa, of which 22 are included in our analysis. This indicates two radiations, one with two species, the other with 11 or 12 taxa. However, morphological data indicate that the first radiation includes eight, and the second includes 20 species. There are possibly more radiations but species sampling would need to be extended to test these. Unfortunately our sampling of the Irideae p.p. of South Central and Eastern Africa is not adequate for a conclusive biogeographical optimisation. The *in situ* diversification in South Central and Eastern Africa contrasts with the situation in the Zimbabwe Overlap Zone, where there has been almost no diversification. Furthermore, in tropical Africa the Restionaceae and the *Pentaschistis* clade also speciated, unlike in the Drakensberg Range. Overall, it seems that there has been more speciation in the geographically much more extensive and fragmented Afrotemperate flora of South Central and Eastern Africa, than in the Drakensberg Range.

CONCLUSION

Biota comprise independent lineages that react differently to barriers, changes in climate, vegetation and pollinators. The biogeographic histories of their components are therefore not necessarily the same even if they occur in the same area. Using well sampled phylogenies the source areas of biota can be identified and the use of molecular clocks further allows us to put these events into a temporal framework. The Cape elements that we investigated occur in the tropical African mountains as a result of migration from the Cape or Drakensberg and also as a result of *in situ* speciation. This is similar to the situation in the Andes, where for the clades investigated the diversity is largely the result of recent and rapid *in situ* speciation (Hughes & Eastwood 2006). We also demonstrate a unidirectional migration in the Afrotemperate flora. In contrast, migration across the Tasman Sea between New Zealand and Australia is bidirectional (Winkworth *et al.* 2002).

We do not attempt to provide a hypothesis for the origin of the complete Afromontane flora, but rather for what is referred to as the Cape element, which nonetheless forms a substantial part of this flora. There has been a lot of migration throughout the region. In some

areas this immigration is the only source of diversity, whereas for other areas *in situ* diversification has been very important. These findings are however lineage dependant. We present overwhelming support for south to north migration for all clades, and show that the Cape element in the Afromontane flora is, at least in part, Cape derived. The Drakensberg Range has played an important role as a stepping-stone in the spread of the flora through this Region. Clades such as *Stoebe*, *Oxalis*, *Cineraria*, *Felicia*, *Ursinia*, *Lobelia*, *Cyphia*, *Cliffortia, Pelargonium* and Phyliceae have, like our study groups, their greatest species richness in the Cape, and probably show a similar pattern. In contrast, *Satyrium*, *Aloe* and *Kniphofia* have most species in the tropical mountains and may show a different pattern. A critical evaluation of the last set of genera would constitute an appropriate test of the generality of our Cape to Cairo hypothesis.

ACKNOWLEDGEMENTS

We thank Vincent Savolainen for the aligned sequence matrix of Irideae p.p., John Manning, Felix Forest and Ben Warren for rbcL sequences, Timo van der Niet and André Olmos Simões for useful discussions, and Mike Pirie and two anonymous reviewers for constructive comments on the manuscript. We also thank the National Research Foundation (South Africa), the National Science Foundation (Switzerland), Stellenbosch University, the University of Zurich and the Swiss Academy of Sciences for funding.

APPENDIX 1

Multidivtime analysis

We followed the protocols described by Rutschmann (2004). The nucleotide frequencies, transition / transversion ratio and rate heterogeneity between the sites for each dataset were calculated with Baseml, implemented in PAML (Yang 1997). These values parameterize the F84 model, which is the most complex model implemented in Multidivtime (Thorne & Kishino 2002). Using this model we calculated the branch lengths using Estbranches, a component of Multidivtime, which were then rate corrected with Multidivtime. The Markov Chain Monte-Carlo was run for 10,000 generations, retaining every 100th sample, after discarding the first 100,000 generations. We repeated this twice, and compared the results to ensure that stationarity had been reached. The dating was calibrated to the mean dates obtained by the global analysis (described below). The standard deviations for rttm and rtrate were set as equivalent to rttm and rtrate. Rttm was set to the estimate age of the basal node, with each time unit equivalent to 10 Myr. Estbranches was used to obtain a tree with estimated branch lengths. From this, we estimated the median amount of evolution between the root and all the tips of the ingroup. Rtrate was set to this amount, divided by rttm (the number of time units from the base to the tip of the tree). Bigtime was set to approximately double the estimated age at the basal node.

Global analysis and calibration

We built a phylogenetic tree for the Angiosperms, in which each study group was represented by two species, selected to span the basal node of the study group. Further species representing other groups as well as nodes for which fossils are available were also included. The topology of the tree was taken from the three-gene Angiosperm phylogeny (Soltis *et al.* 2000). The tree was calibrated by the first occurrence of tricolpate pollen 125 Myr ago (Anderson *et al*. 2005), the first occurrence of African Restionaceae in the 61 Myr old Banke deposits in South Africa (Linder *et al*. 2003), the genistoid legume fossils reported by Lavin *et al*. (2006) from 56 Myr ago, and the estimated age of the genus *Phylica* to 12 Myr based on the age of St Helena (Richardson *et al.* 2001a; Richardson *et al.* 2001b). The tricolpate pollen and the *Phylica* node were used as absolute dates (upper and lower bounds), while the other

two fossil deposits were used as lower bounds only. We used rbcL as “dating gene”, as sequences of this gene were available for all the study groups, and as its semi-clocklike behaviour has been well documented (Gaut *et al.* 1992). The sequences were largely downloaded from Genbank, some were obtained from various researchers working on the Cape flora. The sources are shown in Table 1 of Appendix 1.

The *Disa* tree was additionally calibrated using *D. borbonica*, an endemic of Réunion. An upper age limit of 2 Myr was used, based on the age of Réunion (McDougall 1971). This assumes that speciation occurred as the result of dispersal to Réunion. An underestimation of the age of *D. borbonica* would occur with either of the following two scenarios: If the mainland sister species of *D. borbonica* went extinct; alternatively if *D. borbonica* originated elsewhere, migrated to Réunion and then went extinct in the source area. However, these latter two scenarios are less parsimonious, and unlikely considering the short time-scales.

Table 1. (following page) Sources of the rbcL sequences used for the global analysis.

species	family	source
Carpobrotus edulis	Aizoaceae	F. Forest (unpubl. data)
Psilocaulon parviflorum	Aizoaceae	F. Forest (unpubl. data)
Amaryllis belladonna	Amaryllidaceae	Z69219
Hessea zyheri	Amaryllidaceae	AF116962
Anginon rugosum	Apiaceae	U50222
Heteromorpha trifoliata	Apiaceae	U50227
Arctotheca calendula	Asteraceae	F. Forest (unpubl. data)
Didelta spinosa	Asteraceae	F. Forest (unpubl. data)
Linconia alopecuroides	Bruniaceae	AY490993
Lonchostoma monogynum	Bruniaceae	AY490982
Cotyledon orbiculare	Crassulaceae	F. Forest (unpubl. data)
Crassula perforata	Crassulaceae	AF274594
Acosmium dasycarpum	Fabaceae	U74255
Lotononis galpinii	Fabaceae	Z95538
Podalyria calyptrata	Fabaceae	U74217
Monsonia emarginata	Geraniaceae	L14701
Pelargonium capitatum	Geraniaceae	L14702
Ginkgo biloba	Ginkgoaceae	DQ069500
Spetaea lachenaliiflora	Hyacinthaceae	J. Manning (unpubl. data)
Veltheimia bracteata	Hyacinthaceae	F. Forest (unpubl. data)
Aristea glauca	Iridaceae	AF206736
Bobartia gladiata	Iridaceae	AJ309699
Moraea umbellata	Iridaceae	AJ307149
Watsonia angusta	Iridaceae	AJ309666
Disa spathulata	Orchidaceae	AY368342
Disa tripetaloides	Orchidaceae	AF074151
Endonema retzioides	Penaeaceae	AJ605088
Penaea mucronata	Penaeaceae	AJ605090
Pentameris thuarii	Poaceae	N. Barker (unpubl. data)
Prionanthium dentatum	Poaceae	unpubl. data
Leucodendron laureolum	Proteaceae	U79180
Spatalla curvifolia	Proteaceae	F. Forest (unpubl. data)
Protea repens	Proteaceaeq	U79182
Ranunculus acris	Ranunculaceae	AY395557
Baloskion tetraphyllus	Restionaceae	AF148761
Elegia macrocarpa	Restionaceae	AY881424
Willdenowia arescens	Restionaceae	unpubl. data
Phylica pubescens	Rhamnaceae	Y16769
Trichocephalus stipularis	Rhamnaceae	F. Forest (unpubl. data)
Hemimeris sabulosa	Scrophulariaceae	AF123668
Zaluzianskya katherinae	Scrophulariaceae	AF123662
Halleria lucida	Stilbaceae	AF026828
Retzia capensis	Stilbaceae	Z29669
Stilbe vestita	Stilbaceae	Z68827
Gnidia kraussiana	Thymelaeaceae	AJ295267
Lachnaea villosa	Thymelaeaceae	AJ697804
Heliophila dregeana	Brassicaceae	B. Warren (unpubl. data)
Heliophila digitata	Brassicaceae	B. Warren (unpubl. data)
Heliophila rigidiuscula	Brassicaceae	B. Warren (unpubl. data)
Agathosma ovata	Rutaceae	F. Forest (unpubl. data)

Adenandra uniflora	Rutaceae	AF066803
Calodendron capense	Rutaceae	AF066805

APPENDIX 2

Detailed results for each clade

Irideae p.p.

The root node unambiguously optimises to the Cape (Fig. 1). Internal nodes were optimised with statistical significance to Cape only, unless otherwise shown. Despite widespread distributions of many of the taxa, only two internal nodes were polymorphic, namely the most recent common ancestor (MRCA) of *Moraea muddii* and *M. spathulata*, and of *M. natalensis* and *M. elliotii*. We identify three dispersal events from the Cape to the DR, one dispersal event to SCA and one to the Mediterranean. From one SCA group (the *M. verdickii* grade) there is range expansion into EA (*M. ventricosa* and *M. schimperi*) and the ZOR (*M. schimperi*) and dispersal southwards into the DR (*M. alticola* clade). From the DR this clade expanded northwards into the ZOR (*M. muddii* and *M. spathulata*) and southwards into the Cape (*M. spathulata*). From the second clade of the DR (*M. alpina* clade) there is range expansion into SCA (*M. natalensis* and *M. elliottii*) and into the ZOR (*M. natalensis*). Both *Dietes* and *M. carsonii* represent migration events for which the route cannot be inferred, except to say that they originated in the Cape. Speciation outside of the Cape has occurred in the DR and SCA and in a single case in the ZOR.

Disa

The MRCA of *Disa* is unambiguously traced to the Cape (Figs. 1a and b of Appendix 2). We identify at least five dispersal events out of the Cape (see Table 1): *D. baurii*, into the DR, SCA and ZOR; *Disa borbonica* onto Réunion; the *D. cephalotes* clade into the DR and one, possibly two, dispersal events into the DR represented by the clade comprising sections *Spirales*, *Aconitoideae*, *Micranthae*, and *Emarginatae* plus *D. tysonii*, further referred to as clade ‘z’. Although the MRCA of clade z cannot be optimised to any area, the subtending node optimises unambiguously to the Cape. Lastly, *D. zimbabweensis* occurs in the DR and ZOR and is sister to *D. sagittalis* (Cape and DR). The node subtending these taxa optimises significantly to Cape but also (with moderate support; 0.6) to the DR. It is therefore unclear

whether *D. zimbabweensis* represents migration from the Cape or the DR into ZOR, but this species pair represents at least one migration out of the Cape.

Within clade 'z' one or two dispersal events from the DR northwards can be inferred: the *D. miniata* clade migrated to SCA, and most probably *D. aconitoides* ssp. *goetzeana* migrated to EA. The source area of the Madagascan *D. buchanaviana* (section *Emarginatae*) cannot be resolved; the sub-tending node has low support (0.54) for the DR. There has possibly also been westward dispersal into the Cape by section *Spirales*, but the node joining this clade with *Aconitoideae* and *Micranthae* does not optimise to any area. *D. stairsii* dispersed into EA but the source area is not known. Many *Disa* species occur in more than one area. Several species have expanded their ranges from the Cape (Table 1): *D. tripetaloides*, *D. sagittalis*, *D. lugens* and *D. bracteata* into the DR; ***D. brevicornis*** into DR, SCA and ZOR; and *D. cornuta* into the DR and ZOR. Within the part of section *Stenocarpa* that occurs in the DR there has been range expansion into ZOR and SCA (*D. saxicola*). The vast majority of range expansions however occurred within clade 'z'. From the DR there has been range expansion across the Limpopo River into the ZOR (*D. patula* var. *transvaalensis*, *D. woodii*, *D. fragrans*, *D. rhodantha*, *D. versicolor*), SCA (*D. fragrans*, *D. versicolor*, the *D. perplexa* clade) and westwards back into the Cape (*D. chrysostachya*). The node subtending *D. caffra* (Madagascar, DR and SCA) and *D. hircicornis* (DR, SCA, ZOR and EA) optimises significantly to both DR and SCA, so we cannot establish from where this range expansion occurred. The source areas for *D. aconitoides* ssp. *aconitoides* (Cape and DR), *D. aconitoides* ssp. *goetzeana* (EA) and *D. similis* are probably DR, as optimisations at the subtending nodes receive moderate but not significant support (0.85, 0.84 and 0.84, respectively) for this area only. Within the South-Central African clade of the *Micranthae* there has also been range expansion into EA (*D. erubescens* ssp. *erubescens, D. perplexa* and *D. ochrostachya*), WA (*D. ochrostachya* and *D. perplexa*) and the ZOR (*D. zombica*, *D. erubescens* ssp. *erubescens*, *D. ornithantha*, *D. miniata* and *D. perplexa*). For many of these taxa the precise route of migration cannot be inferred because migration into more than one area has occurred without accompanying speciation.

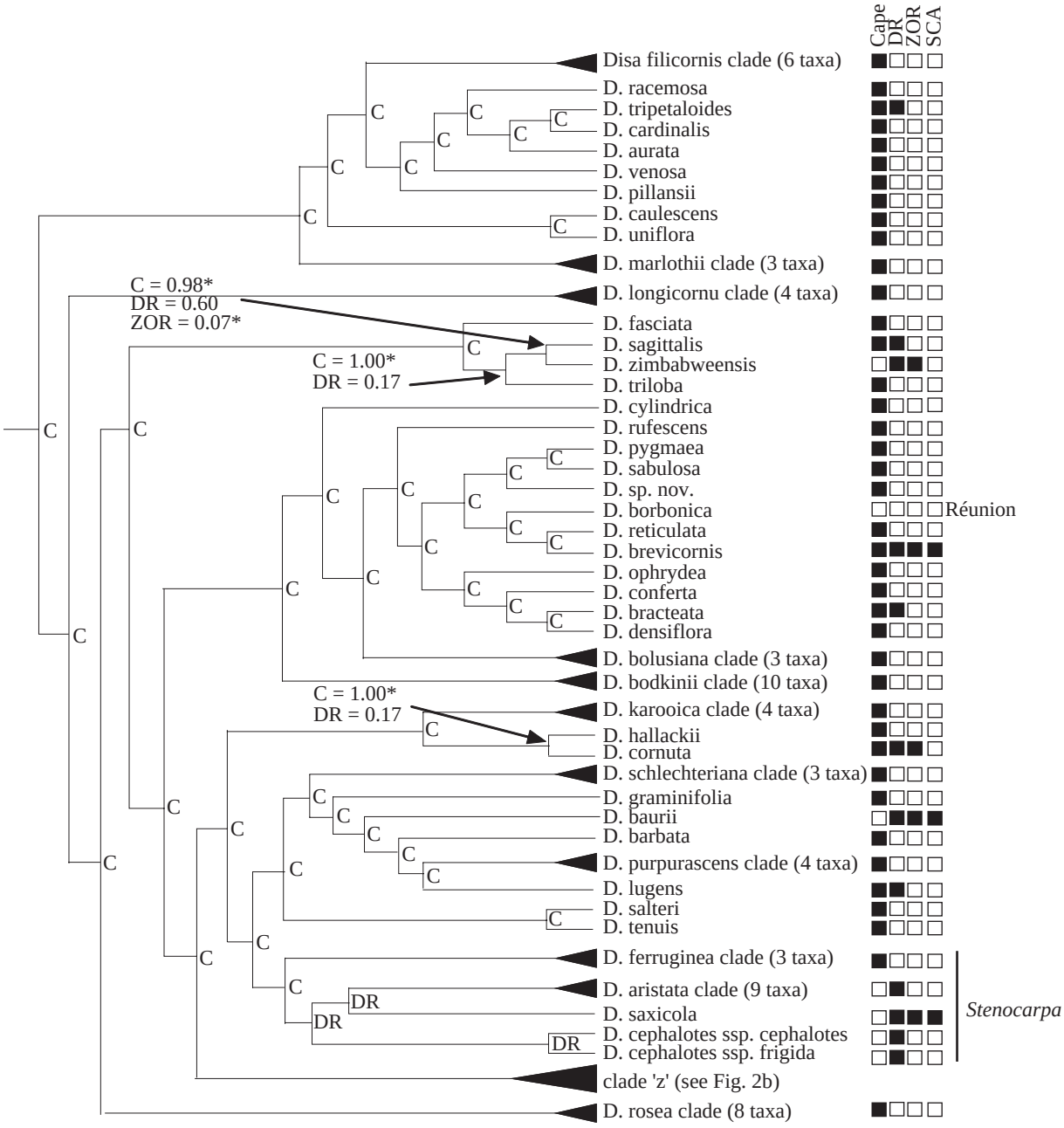
The Pentaschistis *clade*

The root node is unambiguously optimised to the Cape. All internal nodes of the tree are optimised to the Cape, unless otherwise shown in Figure 2 of Appendix 2. We identify eight migration events out of the Cape. A minimum of five of these represent dispersals to the DR (*P. tysonii*, *P. basutorum*, *P. exserta*, *P. chippindalliae* and an event in *P. aurea*) and one a

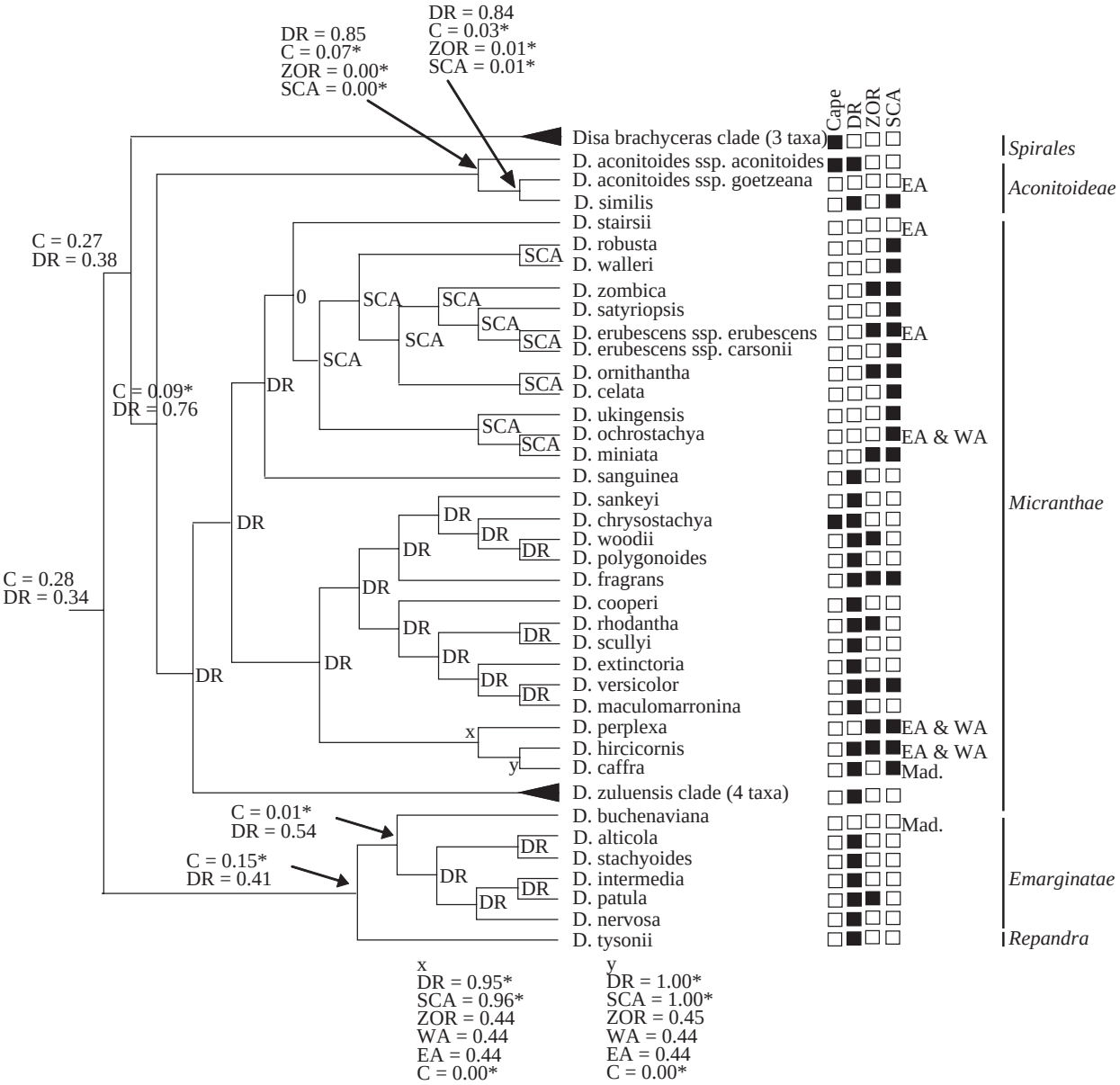
migration to Madagascar (*P. andringitrensis*). The node sub-tending *P. aurea* subsp. *aurea* has proportional likelihoods of 0.78 (Cape) 0.18 (DR) and represents either a vicariance event leading to the two sub-species or (more likely) a single migration into the DR. The node subtending *P. natalensis* from SCA and Madagascar receives no likelihood support, therefore the source area for these cannot be identified. There are several more nodes within the summer rainfall clade that do not optimise to any area. From within this clade migration to EA (the *P. pictigluma* clade) and dispersal to Amsterdam Island (*P. insularis*) occurred, but due to the ambiguous optimisation of the internal nodes, the source area(s) cannot be determined.

Restionaceae

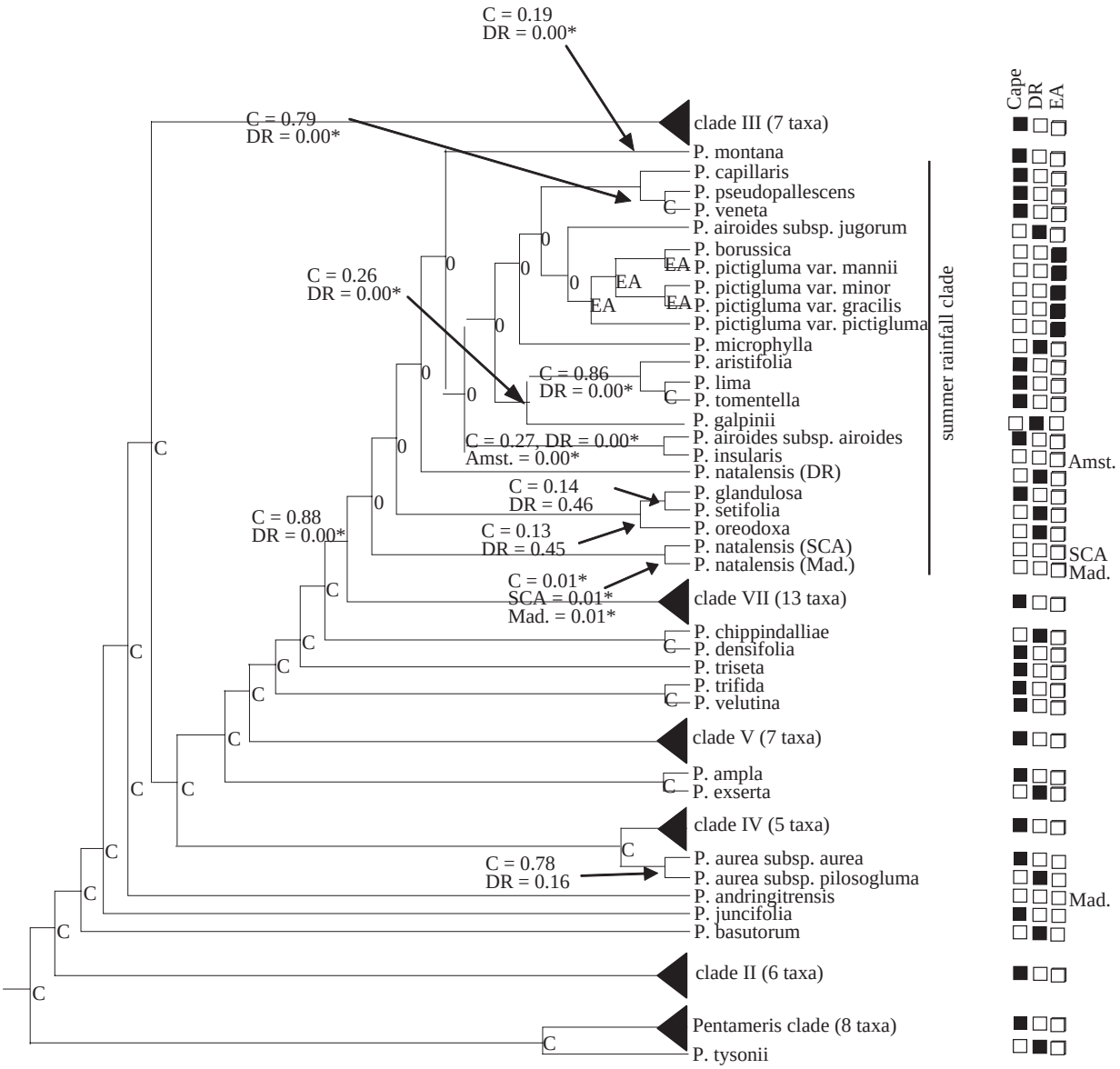
The root node and all internal nodes were unambiguously optimised to the Cape with the exception the *Restio galpinii* clade (see Fig. 3 of Appendix 2). Six or seven migration events out of the CFR are identified: into the DR there are two range expansions (*Rhodocoma fruticosa* and *Restio sejunctus*) and three dispersal events (*Ischyrolepis schoenoides*, *Restio zuluensis*, *Calopsis paniculata*). There is further migration out of the Cape represented by the *Restio galpinii* clade. The MCRA of this clade optimises as absent for all areas, and the node subtending the two species from SCA optimises to SCA. This clade represents one or two migration events out of the Cape. This clade has undergone speciation outside of the Cape, representing one, possibly two speciation events in SCA, or possibly one in the DR.



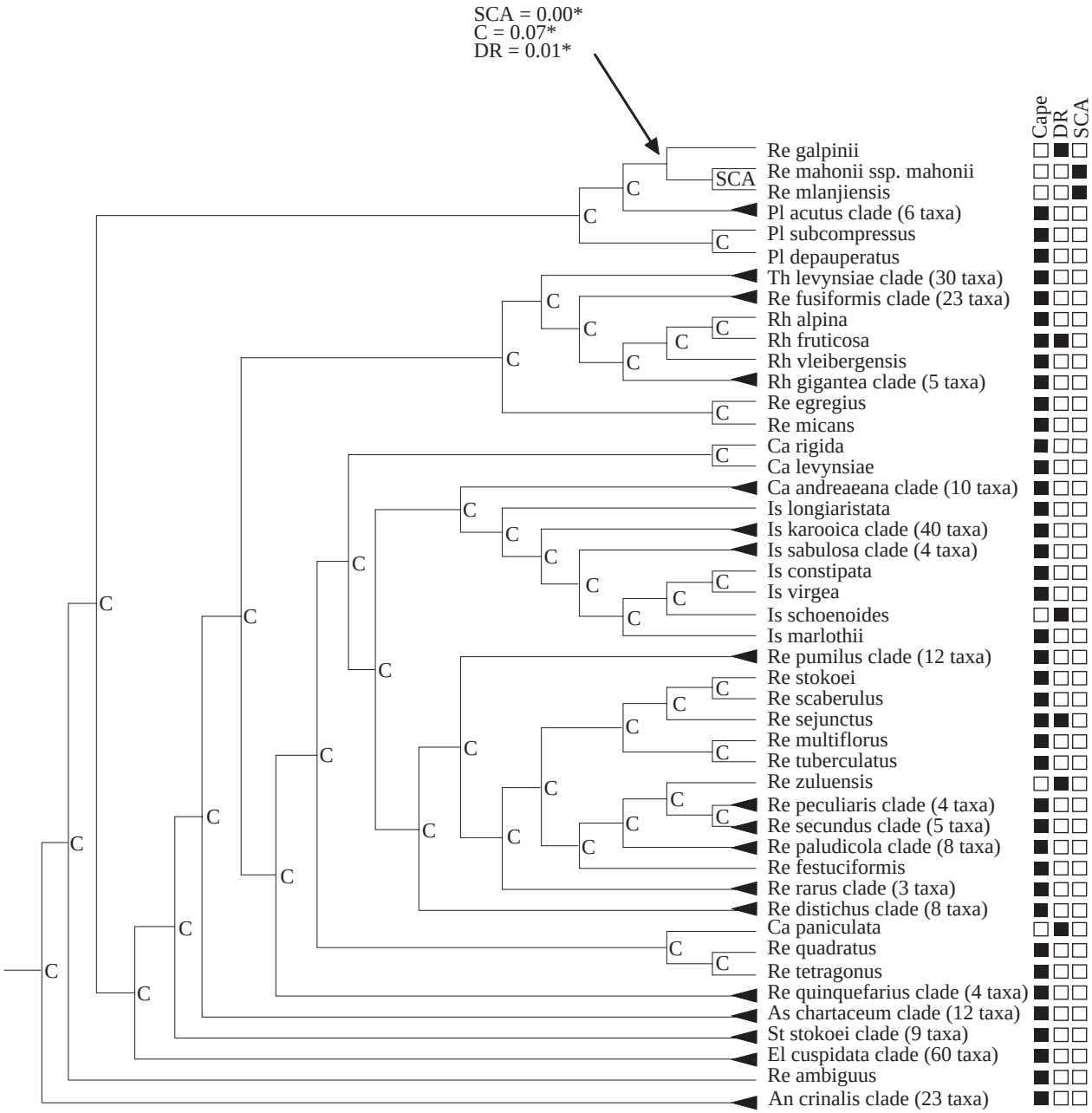
Appendix 2, Figure 1a. Optimisation of ancestral node distribution for *Disa* including proportional likelihoods of areas for nodes that do not optimise unambiguously. clade ‘z’ shown on Figure 1b. Areas as follows: C, Cape; DR, Drakensberg region.



Appendix 2, Figure 1b. Optimisation of ancestral node distribution for clade 'z' of *Disa* including proportional likelihoods of areas for nodes that do not optimise unambiguously. Areas as follows: C, Cape; DR, Drakensberg Region; SCA, South Central Africa; 0, optimises as absent for all areas.



Appendix 2, Figure 2. Optimisation of ancestral node distribution for the *Pentaschistis* clade including proportional likelihoods of areas for nodes that do not optimise unambiguously. Areas as follows: C, Cape; Amst., Amsterdam Island and St. Paul's Island; 0, optimises as absent for all areas. Genus name P is *Pentaschistis*.



Appendix 2, Figure 3. Optimisation of ancestral node distribution for Restionaceae including proportional likelihoods of areas for nodes that do not optimise unambiguously. Area: C, Cape. SCA, South Central Africa. Genera names as follows: An, *Anthochortus*; As, *Askidiosperma*; Ca, *Calopsis*; El, *Elegia*; Is, *Ischyrolepis*; Pl, *Platycaulos*; Re, *Restio*; Rh, *Rhodocoma*; St, *Staberoha*; Th, *Thamnocortus*.

REFERENCES CITED

Adamson, R. S. 1958 The Cape as an ancient African flora. *The Advancement of Science* **58**, 1-10.

Anderson, C. L., Bremer, K. & Friis, E. M. 2005 Dating phylogenetically basal eudicots using *rbc*L sequences and multiple fossil reference points. *American Journal of Botany* **92**, 1737-1748.

Axelrod, D. I. & Raven, P. H. 1978 Late Cretaceous and Tertiary vegetation history of Africa. In *Biogeography and ecology of southern Africa* (ed. M. J. A. Werger), pp. 79-130. The Hague: Dr. W. Junk bv Publishers.

Bonnefille, R., Potts, R., Chalié, F., Jolly, F. & Peyron, D. 2004 High-resolution vegetation and climate change associated with Pliocene *Australopithecus afarensis*. *Proceedings of the National Academy of Sciences of the United States of America* **101**, 12125-12129.

Born, J., Linder, H. P. & Desmet, P. 2006 The Greater Cape Floristic Region. *Journal of Biogeography* **doi: 10.1111/j.1365-2699.2006.01595.x**.

Bytebier, B., Bellstedt, D. U. & Linder, H. P. 2006 A molecular phylogeny for the large African orchid genus Disa. *Molecular Phylogenetics and Evolution* **doi: 10.1016/j.ympev.2006.04.014**.

Carbutt, C. & Edwards, T. 2002 Cape elements on high-altitude corridors and edaphic islands: historical aspects and preliminary phytogeography. *Systematics and Geography of Plants* **71**, 1033-1061.

Carbutt, C. & Edwards, T. J. 2004 The flora of the Drakensberg Alpine Centre. *Edinburgh Journal of Botany* **60**, 581-607.

Cowling, R. M. 1983 Phytochorology and vegetation history in the South-Eastern Cape, South Africa. *Journal of Biogeography* **10**, 393-419.

Craw, R. C., Grehan, J. R. & Heads, M. J. 1999 *Panbiogeography - tracking the history of life*. New York: Oxford University Press.

Crisp, M. D. 2006 Biome assembly: what we know and what we need to know. *Journal of Biogeography* **33**, 1332-1333.

Galley, C. & Linder, H. P. 2006 Geographic affinities of the Cape flora, South Africa. *Journal of Biogeography* **33**, 236-250.

Galley, C. & Linder, H. P. 2007 The phylogeny of the *Pentaschistis* clade (Danthonioideae, Poaceae) based on cpDNA, and the evolution and loss of complex characters. *Evolution* **61**, 864-884.

Gaston, K. J. 2003 *The structure and dynamics of geographical ranges*. Oxford: OUP.

Gaut, B. S., Muse, S. V., Clark, W. D. & Clegg, M. T. 1992 Relative rates of nucleotide substitution at the *rbcL* locus of monocotyledonous plants. *Journal of Molecular Evolution* **35**, 292-303.

Goldblatt, P. 1978 An analysis of the flora of Southern Africa: its characteristics, relationships, and origins. *Annals of the Missouri Botanical Garden* **65**, 369-436.

Goldblatt, P. & Manning, J. C. 2002 Plant diversity of the Cape region of Southern Africa. *Annals of the Missouri Botanical Garden* **89**, 281-302.

Goldblatt, P., Savolainen, V., Porteous, O., Sostaric, I., Powell, M., Reeves, G., Manning, J. C., Barraclough, T. G. & Chase, M. W. 2002 Radiation in the Cape flora and the phylogeny of peacock irises Moraea (Iridaceae) based on four plastid DNA regions. *Molecular Phylogenetics and Evolution* **25**, 341-360.

Griswold, C. E. 1991 Cladistic biogeography of Afromontane spiders. *Australian Systematic Botany* **4**, 73-89.
Grove, A. T. 1983 Evolution of the physical geography of the East African Rift Valley Region. In *Evolution, time and space: the emergence of the biosphere* (ed. R. W. Sims, J. H. Price & P. E. S. Whalley), pp. 115-155. London and New York: Academic Press.
Hardy, C. R. & Linder, H. P. 2005 Intraspecific variability and timing in ancestral ecology reconstruction: A test case from the Cape flora. *Systematic Biology* **54**, 299-316.
Hardy, C. R., Moline, P. & Linder, H. P. submitted A phylogeny for the African Restionaceae and a prospectus for generating complete species phylogenies for large clades.
Hedberg, O. 1965 Afroalpine flora elements. *Webbia* **19**, 519-529.
Hilliard, O. M. & Burtt, B. M. 1987 *The botany of the Southern Natal Drakensberg*. Annals of Kirstenbosch Botanic Gardens. Pretoria: NBI.
Holland, P. G. 1978 An evolutionary biogeography of the genus *Aloe*. *Journal of Biogeography* **5**, 213-226.
Hughes, C. & Eastwood, R. 2006 Island radiation on a continental scale: Exceptional rates of plant diversification after uplift of the Andes. *Proceedings of the National Academy of Sciences of the United States of America* **103**, 10334-10339.
Humphries, C. J. & Parenti, L. R. 1999 *Cladistic biogeography: interpreting patterns of plant and animal distributions*. Oxford: Oxford University Press.
Jürgens, N. 1991 A new approach to the Namib region I: Phytogeographic subdivision. *Vegetacio* **97**, 21-38.
Killick, D. J. B. 1963 An account of the plant ecology of the Cathedral Peak area of the Natal Drakensberg. *Memoirs of the botanical survey of South Africa* **31**, 1-178.
Killick, D. J. B. 1978 The Afro-alpine region. In *Biogeography and ecology of southern Africa* (ed. M. J. A. Werger). The Hague: Dr. W. Junk bv Publishers.
King, L. C. 1963 *South African scenery*. Edinburgh: Oliver and Boyd Ltd.
King, L. C. 1978 The geomorphology of central and southern Africa. In *Biogeography and ecology of Southern Africa* (ed. M. J. A. Werger). The Hague: Dr. W. Junk bv Publishers.
Lavin, M., Herendeen, P. S. & Wojciechowski, M. F. 2006 Evolutionary rates analysis of Leguminosae implicates a rapid diversification of lineages during the Tertiary. *Systematic Biology* **54**, 575-594.
Levyns, M. R. 1938 Some evidence bearing on the past history of the Cape flora. *Transactions of the Royal Society of South Africa* **26**, 404-424.
Levyns, M. R. 1952 Clues to the past in the Cape flora of today. *South African Journal of Science* **49**, 155-164.
Levyns, M. R. 1962 Possible antarctic elements in the South African flora. *South African Journal of Science* **58**, 237-241.
Levyns, M. R. 1964 Presidential address. Migrations and origin of the Cape flora. *Transactions of the Royal Society of South Africa* **37**, 85-107.
Linder, H. P. 1990 On the relationship between the vegetation and floras of the Afromontane and the Cape regions of Africa. *Mitteilungen aus dem Institut für Allgemeine Botanik Hamburg* **23b**, 777-790.
Linder, H. P. 1994 Afrotemperate phytogeography: implications of cladistic biogeographical analysis. In *Proceedings of the XIIIth Plenary Meeting AETFAT, Malawi.*, vol. 2 (ed. A. C. Chikuni), pp. 913-930. Zomba: National Herbarium and Botanic Gardens, Malawi.
Linder, H. P. 2003 The radiation of the Cape flora, southern Africa. *Biological Reviews* **78**, 597-638.
Linder, H. P., Eldenäs, P. & Briggs, B. G. 2003 Contrasting patterns of radiation in African and Australian Restionaceae. *Evolution* **57**, 2688-2702.

Maddison, W. P. & Maddison, D. R. 2006 Mesquite: a modular system for evolutionary analysis. Version 1.11 http://mesquiteproject.org.
McDougall, I. 1971 The Geochronology and evolution of the young volcanic island of Reunion, Indian Ocean. *Geochimica et Cosmochimica Acta* **35**, 261-288.
McGuire, A. F. & Kron, K. A. 2005 Phylogenetic relationships of European and African *Ericas*. *International Journal of Plant Sciences* **166**, 311-318.
Mooers, A. O. & Schluter, D. 1999 Reconstructing ancestral states with maximum likelihood: support for one- and two-rate models. *Systematic Biology* **48**, 623-633.
Nelson, G. & Ladiges, P. Y. 1996 Paralogy in cladistic biogeography and analysis of paralogy-free subtrees. *American Museum Novitates* **3167**, 1-58.
Partridge, T. C. 1998 Of diamonds, dinosaurs and diatrophism: 150 million years of landscape evolution in southern Africa. *South African Journal of Geology* **101**, 167-184.
Partridge, T. C. & Maud, R. R. 1987 Geomorphic evolution of Southern Africa since the Mesozoic. *South African Journal of Geology* **90**, 179-208.
Partridge, T. C. & Maud, R. R. 2000 Macro-scale geomorphic evolution of Southern Africa. In *The Cenozoic of Southern Africa* (ed. T. C. Partridge & R. R. Maud). Oxford: Oxford University Press.
Partridge, T. C., Wood, B. A. & deMenocal, P. B. 1985 The influence of global climatic change and regional uplift on large-mammalian evolution in East and Southern Africa. In *Paleoclimate and evolution with emphasis on human origins* (ed. E. S. Vrba, G. H. Denton, T. C. Partridge & L. H. Burckle). New Haven and London: Yale University Press.
Renner, S. S. 2005 Relaxed molecular clocks for dating historical plant dispersal events. *Trends in Plant Science* **10**, 550-558.
Richardson, J. E., Weitz, F. M., Fay, M. F., Cronk, Q. C. B., Linder, H. P., Reeves, G. & Chase, M. W. 2001a Phylogenetic analysis of *Phylica* L. with an emphasis on island species: evidence from plastid *trnL-F* DNA and nuclear internal transcribed spacer (ribosomal DNA) sequences. *Taxon* **50**, 405-427.
Richardson, J. E., Weitz, F. M., Fay, M. F., Cronk, Q. C. B., Linder, H. P., Reeves, G. & Chase, M. W. 2001b Rapid and recent origin of species richness in the Cape flora of South Africa. *Nature* **412**, 181-183.
Ronquist, F. 1997 Dispersal - vicariance analysis: a new approach to the quantification of historical biogeography. *Systematic Biology* **46**, 195-203.
Rutschmann, F. 2004 *Bayesian molecular dating using PAML/multidivtime. A step-by-step manual*. Zurich: University of Zurich.
Rutschmann, F. 2006 Molecular dating of phylogenetic trees: a brief review of current methods that estimate divergence times. *Diversity and Distributions* **12**, 35-48.
Schluter, D., Price, T., Mooers, A. O. & Ludwig, D. 1997 Likelihood of ancestor states in adaptive radiation. *Evolution* **51**, 1699-1711.
Soltis, D. E., Soltis, P. S., Chase, M. W., Mort, M. E., Albach, D. C., Zanis, M., Savolainen, V., Hahn, W. H., Hoot, S. B., Fay, M. F., Axtell, M., Swensen, S. M., Prince, L. M., Kress, W. J., Nixon, K. C. & Farris, J. S. 2000 Angiosperm phylogeny inferred from 18S rDNA, *rbc*L and *atp*B sequences. *Botanical Journal of the Linnean Society* **133**, 381-461.
Thorne, J. L. & Kishino, H. 2002 Divergence time and evolutionary rate estimation with multilocus data. *Systematic Biology* **51**, 689-702.
Timberlake, J. & Muller, T. 1994 Identifying areas for conservation in Zimbabwe. In *Botanical diversity in Southern Africa* (ed. D. J. Huntley). Pretoria: National Botanical Institute.
Van Wyk, A.-E. & Smith, G. F. 2001 *Regions of floristic endemism in Southern Africa. A review with emphasis on succulents*. Pretoria: Umdaus Press.

Van Zinderen Bakker, E. M. 1978 Quaternary vegetation changes in southern Africa. In *Biogeography and ecology of Southern Africa* (ed. M. J. A. Werger). The Hague: Dr. W. Junk bv Publishers.
Vincens, A., Tiercelin, J.-J. & Buchet, G. 2006 New Oligocene-early Miocene microflora from the southwestern Turkana Basin; palaeoenvironmental implications in the northern Kenya Rift. *Palaeogeography, Palaeoclimatology, Palaeoecology* **239**, 470-486.
Weimarck, H. 1936 Die Verbreitung einiger Afrikanisch-montanen Pflanzengruppen, III-IV. *Svensk Botanisk Tidskrift* **30**, 36-56.
Weimarck, H. 1941 Phytogeographical groups, centres and intervals within the Cape flora. *Lunds Universitets Arsskrift* **37**, 1-143.
White, F. 1978 The Afromontane region. In *Biogeography and ecology of Southern Africa* (ed. M. J. A. Werger). The Hague: Dr W. Junk bv Publishers.
White, F. 1983 *The vegetation of Africa*. Paris: Unesco.
Wild, H. 1964 The endemic species of the Chimanimani Mountains and their significance. *Kirkia* **4**, 125-157.
Wild, H. 1968 Phytogeography in South Central Africa. *Kirkia* **6**, 197-222.
Winkworth, R. C., Wagstaff, S. J., Glenny, D. & Lockhart, P. J. 2002 Plant dispersal NEWS from New Zealand. *Trends in Ecology & Evolution* **17**, 514-520.
Wulff, E. V. 1950 *An introduction to historical plant biogeography*. Waltham, Massachusetts, U.S.A.: Chronica Botanica Company.
Yang, Z. 1997 PAML: a program package for phylogenetic analysis by maximum likelihood. *CABS* **13**, 555-556.

End summary

This thesis is a macro-evolutionary and macro-ecological study of a species rich group of grasses from the Cape flora (the *Pentaschistis* clade), and other clades which share its distribution. The first two chapters are papers investigating morphological evolution and ecological diversity of the *Pentaschistis* clade. Chapter three is a description of new species. Chapters four and five are meta-analyses of the historical biogeography of multiple lineages, including the *Pentaschistis* clade; the former investigating the origins of a big component of the Cape flora and the latter focussing on the assembly of diversity in some Afrotemperate floras.

The species tree and character evolution

In chapter one I investigated the evolution and function of multicellular glands and leaf anatomical types in the *Pentaschistis* clade. These characters are correlated but it is unclear how many times each evolved or was lost. Studying character evolution requires a densely sampled, species-level phylogenetic hypothesis of the group. Chloroplast DNA sequences of four gene regions were analysed using Bayesian inference and reconstructed ancestral morphological character states using likelihood optimisation. I investigated the leaf anatomical background with which glands evolved, were gained or were lost, and with which character combination diversification did or did not occur.

Glands evolved once or twice in the *Pentaschistis* clade. Conversely leaf anatomy type is fairly labile. Although glands are lost several times from orthophyllous-leaved plants, these lineages do not diversify; lineages of glandless plants only diversify when the plants are sclerophyllous-leaved. Most diversification occurred in lineages of sclerophyllous-leaved glandless plants, and in lineages with orthophyllous-leaved glanded plants. The hypothesis which is developed to explain these patterns of diversification is that either glands or sclerophyllous leaves act as defence systems against herbivory, permitting lineage diversification. Having neither defence strategy precludes diversification, and having both strategies is a costly, unstable combination. Secondly, leaf anatomical type is linked to nutrient poor or rich soils. Diversification of sclerophyllous lineages is restricted to nutrient poor soils, whereas lineages of orthophyllous type evolve onto and diversify on both soil types. This suggests that the evolution of glands allowed orthophyllous taxa to persist and to diversify onto both types of soil. Lastly, some issues regarding inter-generic relationships are resolved. *Prionanthium* is nested within *Pentaschistis* and this clade is sister to *Pentameris* plus *Pentaschistis tysonii*.

Ecology

Chapter two investigated the species richness of the *Pentaschistis* clade in the Cape Floristic Region, using the Drakensberg region as a comparison. We searched for correlations between landscape heterogeneity and turnover with species richness and turnover. Five environmental variables were combined and used to characterise the species and the landscapes of both regions.

The amount of habitat available in the Cape and Drakensberg is not significantly different, but importantly, the amount of habitat estimated as suitable for *Pentaschistis* is much higher in the Cape. Furthermore, we show that species in the Cape overlap more ecologically, and suggest that more diverse micro habitats might enable more species to co-exist in a landscape in the Cape. The turnover of species between landscapes cannot be

explained simply by habitat turnover. Species in the Cape are range restricted for a number of reasons, included spatially restricted niches, and distributional disequilibrium, as estimated by predictive modelling. Our method of categorising the habitats in the two regions is coarse, but this is a suitable approach when the dataset for a region contains some sparsely sampled species, as is the case for many species rich areas.

New species description

Despite being a well collected flora, the discovery and description of new species from the CFR are continuing. The description of three new species from the CFR (*Pentaschistis clavata*, *P. rigidissima* and *P. trifida*) adds to this. Specimens of *P. rigidissima* were previously included in *P. horrida* but morphological differences remain when the two species occur in sympatry. This is backed up by phylogenetic analysis of molecular data. A fourth species, *P. juncifolia*, is resurrected. It was previously included in another species but there are consistent morphological and ecological differences between the species, and the distinction is again supported by molecular data.

The origins of the Cape flora

A flora does not have a single origin, it has origins, which are those of its component lineages. Chapter four focussed on the origins of the 'Cape clades', which represent ~50% of the species in the Cape flora. Using clades rather than taxa allows us to be more precise, and sometimes more accurate in defining these mega species-rich groups in the Cape. Published and unpublished phylogenies based on molecular and morphological data were used to identify the sister group to the clade of interest, and to estimate the relationships in the earlier branching events within each clade. In many cases this allowed us to estimate from where the lineage had arrived into the Cape, and a general pattern across all the clades was searched for. Additionally, secondary calibration points were used where possible, to estimate the ages of when the Cape lineages split from their sister group.

The Cape floral clades have very different origins, both in space and over time. Lineages have been sourced especially from Tropical Africa, Australia and Europe. The sourcing of lineages from tropical Africa is not as important as was suggested by previous authors, as in many cases the tropical African species are nested within the Cape clade. Conversely recruitment from Australia has been more frequent than had been suggested. Although the error in the dating estimates is large it is clear that recruitment into the Cape has occurred over a wide range of time. From reviewing the published literature it is clear that much more sampling is needed to complete species-level phylogenetic analyses of Cape clades, especially regarding the non-Cape species. This chapter therefore represents an initial meta-analysis.

The Cape element in the Afrotemperate flora

Chapter five investigates the migration routes of Cape centred lineages through the Afrotemperate region, which were until now poorly known. The CFR is known to be a region where many plant lineages have diversified, but we know much less about diversification in other areas; local diversity may be due to in situ diversification or immigration to the area. This was tested by optimising geographical distribution onto internal nodes of the phylogenetic trees of four lineages: *Disa*, Irideae p.p., the *Pentaschistis* clade and the African Restionaceae.

We identify a general pattern of migrations around the Afrotemperate Region. These clades all have their origins in the CFR. Almost all migrations into the East African

mountains are sourced in the Drakensberg or the Malawi / Zimbabwe regions; there are very few migrations directly from the Cape. This suggests that the Drakensberg has played an important role in the recruitment of lineages from the Cape to the tropical Afromontane region. Migrations to the tropical Afromontane region occurred relatively recently, within the last 15 mya. The Cape to Drakensberg route is an important migration route which is dated to as early as 25.65 (17.25-34.80 credibility interval) mya ago. The amount of in-situ diversification in different areas of the Afrotemperate region is partly lineage dependant. In addition to the CFR, secondary centres of diversity include the Drakensberg (for *Disa* and Irideae p.p.), the East African mountains (for *Pentaschistis*) and southern central Africa (for *Disa* and possibly Irideae p.p.). We identify no local diversification in the Zimbabwe Overlap Region. Lastly, we propose a list of taxa that should be investigated in a similar manner to test the generality of our hypothesis of plant migration throughout the Afrotemperate flora.

Zusammenfassung

Die vorliegende Arbeit behandelt die Makroevolution und die Makroökologie einer artenreichen Gruppe von Grasen der Kapflora (der *Pentaschistis* „Clade“), und anderer „Clades“, welche dieselbe Verbreitung aufweisen.

Die ersten zwei Kapitel bestehen aus Artikeln, welche Aspekte zur morphologischen Evolution und ökologischen Diversität des *Pentaschistis* „Clade“ untersuchen. Kapitel drei ist eine Beschreibung neuer Pflanzenarten. Kapitel vier und fünf enthalten Meta-Analysen zur historischen Biogeographie mehrerer Abstammungslinien, darunter des *Pentaschistis* „Clade“; ersteres untersucht die Abstammung eines grossen Teils der Flora am Südafrikanischen Kap, letzteres betrachtet die Zusammensetzung der Florendiversität in einigen Regionen der gemässigten Zonen Afrikas.

Stammbaum der Arten und Merkmalevolution

Im ersten Kapitel untersuche ich die Evolution und Funktion von mehrzelligen Drüsen und von anatomischen Blatttypen in der *Pentaschistis* Gruppe. Diese Merkmale sind miteinander korreliert, aber es ist nicht bekannt, wie oft sie evoluiert haben oder wieder verloren gegangen sind. Um Merkmalsevolution zu untersuchen, benötigt man eine phylogenetische Hypothese der Gruppe auf Artniveau, welche sich auf einem gut aufgelösten Datensatz abstützt. Dafür wurden DNA Sequenzen von vier Regionen des Chloroplastengenoms mittels Bayes-Inferenz analysiert und die ursprünglichen morphologischen Merkmalszustände mittels „likelihood“-Optimierung rekonstruiert. Ich habe untersucht, auf welchen anatomischen Grundlagen die genannten Drüsen sich entwickelt haben, ob sie mehrmals evoluiert haben oder aber verloren gegangen sind, und mit welchen Merkmalskombination eine Diversifikation stattgefunden hat.

Drüsen evoluierten nur einmal oder zweimal im *Pentaschistis* „Clade“, hingegen ist der anatomische Blatttypus ein ziemlich labiles Merkmal. In orthophyll-blättrigen Pflanzen sind die Drüsen mehrere Male verloren gegangen, allerdings haben die betreffenden Abstammungslinien nie diversifiziert; in Linien ohne Drüsen haben interessanterweise nur Planzen vom sklerophyll-blättrigen Typus diversifiziert: die grösste Diversifikation fand in Pflanzenlinien vom Typ drüsenlos/sklerophyll-blättrig bzw. bedrüst/orthophyll-blättrig statt. Gemäss der aus diesen Beobachtungen entwickelten Hypothese sind entweder Drüsen oder sklerophyllartige Blätter Teil eines Abwehrsystems gegen Herbivore, welches einer Abstammungslinie die Diversifikation erlaubt. Keines dieser beiden Abwehrsysteme zu besitzen hätte eine Pflanzengruppe an der Diversifikation gehindert; andererseits mag es kostenungünstig und daher unstabil sein, beide Abwehrsysteme zu besitzen. Der anatomische Blatttypus steht ausserdem in direktem Zusammenhang mit dem Nährstoffgehalt des Bodens. Diversifikation von sklerophyll-blättrigen Linien ist auf nährstoffarme Böden beschränkt, wohingegen orthophyll-blättrige Linien auf beide Bodentypen evoluiert und dort diversifiziert haben. Die Evolution von Drüsen scheint es also orthophyll-blättrigen Taxa ermöglicht zu haben, auf beiden Bodentypen zu bestehen und zu diversifizieren.
Schliesslich werden einige unklare Punkte betreffend zwischenartlichen Beziehungen aufgelöst. *Prionanthium* ist innerhalb *Pentaschistis* anzusiedeln und dieser „Clade“ kann als Schwestergruppe zu *Pentameris* und *Pentaschistis tysonii* betrachtet werden.

Neue Artbeschreibung

Trotz einer gut untersuchten Flora werden immer noch neue Arten von der Region der Kapflora (CFR) entdeckt und beschrieben. Hierzu kommt die Beschreibung dreier neuer Arten der CFR: *Pentschistis clavata*, *P. rigidissima* und *P.trifida*. Exemplare von *P. rigidissima* wurden früher in *P. horrida* eingeschlossen, aber morphologische Unterschiede bleiben bestehen, auch wenn die beiden Arten sympatrisch auftreten. Diese Beobachtungen werden von phylogenetischen Analysen mittels molekularer Daten unterstützt. Eine vierte Art, *P. juncifolia*, wird wieder eingeführt. Sie wurde einer anderen Art zugeordnet, aber es gibt konsistente morphologische und ökologische Unterschiede zwischen den Arten. Auch hier wird die Unterscheidung durch molekulare Daten gestützt.

Die Ursprünge der Kap Flora

Eine Flora besitzt nicht einen einzelnen Ursprung, sondern mehrere; sie ergeben sich aus den Ursprüngen der einzelnen Abstammungslinien der Flora. Kapitel vier konzentriert sich auf die Ursprünge der „Cape Clades“, welche ca. 50% der am Kap vorkommenden Pflanzenarten ausmachen. Indem wir ganze „Clades“ anstelle von Taxa betrachten, können wir die äusserst artenreichen Pflanzengruppen des Kaps viel präziser und oft auch zutreffender definieren. Veröffentlichte und unveröffentlichte Phylogenien - abgestützt auf molekularen und morphologischen Daten - wurden benutzt um Schwesterngruppen zu den untersuchten „Clades“ zu identifizieren, und um die Verwandtschaftsverhältnisse aus früheren Verzweigungsereignissen innerhalb der einzelnen „Clades“ abzuschätzen. Dies erlaubte uns oft eine Abschätzung, woher die Abstammungslinie das Kap erreichte, und es wurde nach generellen Mustern über die verschiedenen „Clades“ gesucht. Zusätzlich wurden, wenn immer möglich, sekundäre Kalibrationspunkte zur Schätzung dafür benutzt, wann sich die Abstammungslinien des Kaps von ihren Schwestergruppen getrennt haben.
Die „Cape Clades“ haben räumlich und zeitlich sehr unterschiedliche Ursprünge. Sie stammen hauptsächlich aus dem tropischen Afrika, Australien und Europa. Das tropische Afrika spielt als Herkunftsort für die Kapflora eine kleinere Rolle als dies in früheren Berichten erwähnt wurde, denn die Arten aus dem tropischen Afrika sind oft in den „Cape Clades“ eingebettet. Hingegen fand eine Rekrutierung von Arten aus Australien in grösserem Ausmass statt als bisher angenommen wurde. Trotz einigen Unsicherheiten in den geschätzten Datierungen wird klar, dass eine Immigration von Arten über einen grossen Zeitraum hinweg stattgefunden haben muss. Bei Betrachtung der publizierten Daten wird ausserdem klar, dass mehr Daten gesammelt werden müssen um die Phylogenie der Kap „Clades“ zu vervollständigen, speziell gilt dies auch für die Flora ausserhalb des Kaps. Dieses vierte Kapitel repräsentiert deshalb eine erste Metaanalyse.

Das „Cape element“ in der Flora der gemässigten Zones Afrikas (Alternativ: Elemente des Kaps in der Flora des gemässigten Afrikas)

Kapitel fünf untersucht die Wanderungsrouten von Linien, welche hauptsächlich am Kap beheimatet sind, durch die Regionen des gemässigten Afrikas. Über diese Routen weiss man bisher nur wenig. Die CFR ist bekanntlich eine Region in welcher viele Pflanzenlinien diversifiziert haben, doch es ist nur wenig bekannt über die Diversifikation in anderen Gebieten; lokale Diversität könnte durch Immigration oder aber Diversifikation *in situ* zustande kommen. Dies wurde durch eine Abschätzung der geographischen Verteilung auf die internen Knotenpunkte des phylogenetischen Stammbaums von vier Arten getestet: *Disa*, Irideae p.p., die *Pentaschistis*-Gruppe und die Afrikanischen Restionaceae.

Wir haben ein generelles Migrationsmuster um die Region des gemässigten Afrikas identifizieren können. Beinahe alle Migrationsbewegungen in das Ostafrikanische Gebirge gehen von den Drakensbergen oder der Malawi/Zimbabwe Region aus. Es scheint also, dass die Drakensberge einen wichtigen Ausganspunkt für die Migration von Abstammungslinien vom Kap in die tropische Afromontane Region gespielt haben. Die Wanderungen in die tropische Afromontane Region haben erst kürzlich stattgefunden, schätzungsweise innerhalb der letzten 15 Millionen Jahre. Die Route vom Kap in die Drakensberge stellte eine wichtige Migrationsroute dar, welche bis auf 25.65 Millionen Jahre (Vertrauensintervall 17.25-34.80 Mio. Jahre) zurückdatiert werden kann. Das Ausmass lokaler Diversifikation in verschiedenen Regionen hängt von der Abstammungslinie ab. Zusätzlich zur CFR können sekundäre Diversifitätszentren wie die Drakensberge (für *Disa* und Irideae p.p.), das Ostafrikanische Gebirge (für *Pentaschistis*) und das südliche Zentralafrika (für *Disa* und möglicherweise Irideae p.p.) genannt werden. Wir haben keine lokale Diversifikation in der „Zimbabwe Overlap Region“ identifizieren können. Schliesslich führen wir eine Liste von Taxa auf, welche auf ähnliche Art und Weise untersucht werden sollten, um die generelle Gültigkeit unserer Hypothese zur Pflanzenmigration innerhalb der Flora des gemässigten Afrika zu überprüfen.

Curriculum Vitae

Personal

Surname:	GALLEY
Name:	Chloé
Date of birth:	13th August 1980
Nationality:	British and French

Education

Secondary school:	Eggar's School, Alton, Hampshire (1991-1996). General Certificate of Secondary Examinations.
Sixth form college:	Alton College, Hampshire (1996-1998). A-levels
Undergraduate university:	Bachelor of Arts in Biology, University of Oxford (1998-2001)
Postgraduate university	Masters in Biodiversity and Taxonomy of Plants, University of Edinburgh (2001-2002)
Master's thesis:	Maternal gene flow in *Quercus petraea* and *Q. robur*.
Ph.D. thesis:	Macro-evolutionary and macro-ecological studies on the Cape flora, with focus on the *Pentaschistis* clade (Poaceae). Employed as a PhD student since November 2002.